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(Recognosce notum, ignotum inspice)

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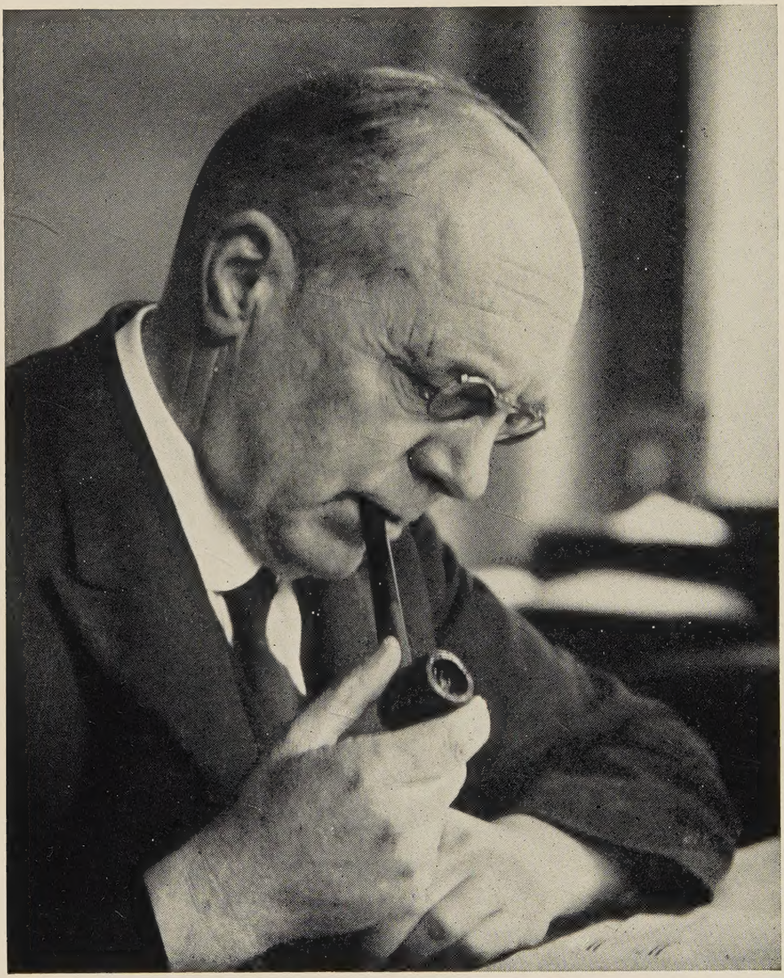

F. T. BROOKS

OBITUARY NOTICE

PROFESSOR F. T. BROOKS, C.B.E., M.A., F.R.S.

Frederick Tom Brooks was born at Wells on 17 December 1882. He received his early education at Sexey's School, Bruton, where a keen interest in field botany was inspired and encouraged by his headmaster. Later he spent several years at a training college for teachers in Bristol, before going up to Emmanuel College, Cambridge, as an Exhibitioner in 1902. At Cambridge he obtained first-class honours in both parts of the Natural Sciences Tripos and was awarded the Frank Smart Studentship. By this time he had come under the influence of two personalities destined to play a big part in stimulating his interests and shaping his future. Marshall Ward, then Professor of Botany, aroused in him a desire to study fungi and plant diseases, and made him a demonstrator in the Botany School. His first piece of original work concerned the mode of infection by conidia of *Botrytis cinerea* Fr., but before he had completed it Marshall Ward died and Brooks, still only twenty-four, was left to take over the teaching of mycology and plant pathology. Though so soon bereft of one guiding hand, he found another in R. H. (later Sir Rowland) Biffen, who was eight years his senior. These two had common interests in mycology, in the countryside and in gardening, and there sprang up between them a close friendship that lasted until Biffen's death forty years later.

The strongly practical cast of Brooks's mind soon became evident both in his teaching and in his research. He began a laboratory study of the life history and nuclear behaviour of *Gnomonia erythrostoma* (Fr.) Auersw., but his urge towards field work led him into the cherry orchards of Kent, where Leaf Scorch caused by this fungus was then epidemic. He also spent much of his time making observations in the fruit-growing districts of Cambridgeshire, and it was here that he began his investigations of Silver Leaf disease, which continued off and on for more than twenty-five years, and which established his reputation as a highly competent researcher into essentially practical problems.

In 1913 he was seconded for a year to the Federated Malay States, where he studied a number of diseases of rubber trees, notably Pink Disease (*Corticium salmonicolor* Berk. & Br.) and a root disease caused by *Ustulina zonata* (Lév.) Sacc. After returning to England just before the outbreak of the 1914–18 War, he sought to enlist in the armed forces, but was not accepted because of bad eyesight. His desire to take an active part in the national emergency was very strong, however, and when, early in 1917, a new Food Production Department was set up, he joined the staff as Plant Pathologist. Much of his time during the next two years was spent in organizing spraying campaigns against potato Blight and in carrying out field observations on this disease.

Brooks returned to Cambridge in 1919 as University Lecturer in Botany. He was a fluent lecturer with the capacity to stimulate and inspire young

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people. Consequently, there were always one or two of his own students wanting to study fungi. Before long, post-graduate workers from other British universities and from overseas also began to flock to the Botany School at Cambridge to work under him, and as time went on senior workers came from many parts of the world, if only for a few months, to gain the benefit of his experience and advice. A generous gift made to the University by the Rockefeller Foundation in or about 1930 included a large sum for the building and equipment of a modern department of plant pathology. This gave Brooks ample scope to develop his ideas. On three acres of land acquired at the University Farm he built a small field laboratory with glasshouse accommodation, and planted the rest with a selection of economic and ornamental trees, shrubs and herbaceous plants. He also initiated a new course of lectures and laboratory work on bacteria and bacterial diseases. In all, Brooks was responsible for training about one hundred specialists in mycology and plant pathology during the forty years he taught at Cambridge, and as a teacher there is no doubt he exerted a profound influence on the development and progress of botany and plant pathology both here and overseas.

When he returned to Cambridge Brooks also resumed and intensified his research on Silver Leaf disease, then rampant in the country as a result of neglect in orchards during the war years. He was able to call on the full-time assistance of a succession of his post-graduate students, and with the aid of a generous grant from the Development Commission built a new greenhouse on the University Farm and rented for some years a small commercial plantation of young plum trees at Meldreth, on which a very wide range of experiments was carried out.

Much of Brooks's published original work was done in collaboration with his research students, and apart from a study of mould growths developing on meat in cold storage, it was concerned mainly with crop diseases of economic importance, such as silver leaf, apple Bark Canker (*Phacidiella discolor* (Mouton & Sacc.) Potebn.), willow Scab (*Fusicladium saliciperdum* (Allesch. & Tub.) Tub.), Black Stem (*Ascochyta imperfecta* Peck) of lucerne, and Canker and Die Back (*Griphosphaeria corticola* (Fuckel) Höhnel) of roses. His friendship with Biffen led him to take a special interest in cereal rusts, and for over twenty years he was engaged in personal research on the rusts of brome grasses, but unfortunately it was left unfinished and none of it has been published. In 1928 he collaborated with the late Sir John Fryer in a book on *Insect and Fungus Pests of the Farm* and the same year published his well-known text-book *Plant Diseases*. A second enlarged and completely rewritten edition of this was finished shortly before his death and appeared posthumously in February 1953. From 1927 onwards he also edited and revised various editions of the two volumes of D. H. Scott's *Introduction to Structural Botany*.

In 1931 Brooks had been appointed to a newly created University Readership in Mycology, and five years later he succeeded Sir Albert Seward as Professor of Botany. He became a Fellow of the Royal Society in 1930 and Fellow of Emmanuel College the following year: he was also an Honorary Fellow of the Royal Society of Edinburgh and the Botanical Society of Edinburgh, and an Honorary LL.D. of the University of

St Andrews. He retired in 1946 with the title of Emeritus Professor of Botany and was awarded the C.B.E. in the 1947 New Year Honours List.

Brooks showed administrative ability of a high order and many demands were made on his services. He was very influential in the counsels of a number of scientific bodies and took a particular interest in the development of our own Society. He was Chairman of the Plant Pathology Committee from 1919 to 1931, President in 1922, and an Honorary Member from 1948. When his busy life permitted, and especially in earlier years, he found much pleasure in attending the autumn forays. He was President of Section K (Botany) at the Norwich meeting of the British Association in 1935 and for the next eleven years served as one of the General Secretaries of the Association. From 1945 to 1947 he was President of the Cambridge Philosophical Society.

He represented the University of Cambridge on the governing bodies of several of our older agricultural and horticultural research stations, and became the first Chairman of Governors of the recently established National Vegetable Research Station. He served two terms of office as a member of the Agricultural Research Council and took a very prominent part in the many-sided activities of that body, particularly after his retirement from the Chair of Botany.

Despite his many activities and busy life Brooks never lost his innate interest in the countryside or his love for Somerset. His ruddy countenance, robust figure and marked west-country 'burr' bore evidence of this, and among the many honours he received he cherished none more than his appointment in 1936 as a Governor of his old school. Brooks worked with zest even at unpalatable tasks. He was resolute and determined, self-reliant, and thorough in all he did, and his shrewd common sense, clear thinking and honesty of purpose appealed to everyone. Always partial to games, he continued to play lawn tennis and squash rackets until well into his fifties, and was very keen on swimming. These vigorous pursuits, combined with an inherent robustness and fondness for walking and gardening, kept him physically fit and able to cope with a prodigious amount of work. His forceful and forthright character tended to frighten his students at first but they soon came to realize that superficial severity was pleasantly tempered by great friendliness and geniality, and sooner or later timidity gave way to affectionate respect and later, in many instances, to warm friendships.

A severe attack of bronchitis in the spring of 1950 left Brooks with a much weakened heart. He appeared to make a complete recovery, but he died suddenly at his home in Cambridge during the evening of 11 March 1952 in his seventieth year. In 1907 he married Emily, daughter of Mr David Broderick: there were no children of the marriage. W. C. MOORE

A FURTHER COMPARATIVE STUDY OF PATHOGENIC ISOLATES OF *VERTICILLIUM*: *V. NUBILUM* PETHYBR. AND *V. TRICORPUS* SP.NOV.

By IVOR ISAAC

University College of Swansea

(With Plate 10 and 7 Text-figures)

The morphology, physiology, and pathogenicity of two groups of isolates of *Verticillium* are described and compared with *V. albo-atrum*, *V. dahliae* and *V. nigrescens*. The chlamydospore-forming isolates are referred to *V. nubilum* Pethybr., while those which develop microsclerotia, resting mycelium, and chlamydospores simultaneously, are referred to *V. tricorpus* sp.nov.

The optimum temperature for growth of *V. nubilum* was 20–22·5° C. and the optimum pH 7·2–8·6. Growth was maximal on sucrose or dextrose, and was good on maltose and glycerine media. With different nitrogen sources growth was best on media containing either sodium nitrate, buffered ammonium sulphate, 0·1% asparagine, or 0·1% peptone. *V. nubilum* was pathogenic only to potato (Eclipse) and tomato (Kondine Red), although re-isolates were obtained from apparently healthy inoculated antirrhinum plants.

V. tricorpus, which showed somewhat similar temperature, pH, and carbohydrate requirements, grew best with buffered ammonium sulphate as the nitrogen source and was pathogenic to tomato only of the hosts tested.

INTRODUCTION

I have shown in an earlier paper (Isaac, 1949) that the morphology of resting bodies of certain isolates of *Verticillium* is sufficiently constant to be of some importance in determining the classification of this genus. On a wide range of artificial culture media *V. albo-atrum* Reinke & Berthold forms black resting mycelium or 'Dauermycelien' only, *V. dahliae* Kleb., microsclerotia only, and *V. nigrescens* Pethybr. chlamydospores only. Studies of the physiology and pathogenicity of these types also forced me to the conclusion that they are distinct forms, and that the contention that all of them should retain the specific rank originally accorded them is fully justified. I propose now to present evidence from cultural and phytopathological experiments to show that there are still another two species of *Verticillium* causing wilt of tomato plants; the first producing chlamydospores only; the second forming all three types of resting bodies—resting mycelium, microsclerotia and chlamydospores—simultaneously, and when this fungus is grown on artificial media hyaline sectors frequently appear but variants producing only one or two of the resting bodies never arise.

Pethybridge (1919), working on potato diseases in Ireland, discovered two chlamydospore-forming species of *Verticillium* growing on the surface of potato tubers: *V. nigrescens* Pethybr. and *V. nubilum* Pethybr. The only other reference in the literature to *V. nubilum* is that of Gilman (1945), who merely stated that this fungus was a species of uncertain position. Pethybridge stated that the chlamydospores of his *V. nubilum* 'are more or less spherical cells, hyaline at first but soon developing thickish, dark brown or black walls'. These chlamydospores were considerably larger (diameter 8·5–12 μ) than those of *V. nigrescens* (7–10 μ); they were borne singly or in small groups and were terminal or intercalary in rows of three or four or more. In those of my isolates which produced chlamydospores only, these appeared in terminal and intercalary positions and they remained viable for at least 6 years on agar-slants in test-tubes. Since, as shall be seen, these strains resemble *V. nubilum* Pethybr. in many respects, I propose to retain that name.

I have failed to find any reference in the literature to a species of *Verticillium* forming all three types of resting structure, although it is possible that this species has been observed by other workers and, owing to the occurrence of microsclerotia, has been described as *V. dahliae*. Van Beyma Thoe Kingma (1939–40) described certain fungi which grew very much like *V. dahliae* Kleb. and formed chlamydospores and 'pseudosclerotia' on potato, oat-meal and malt-agar media. Examination of his fungi, subcultures of which were obtained from Baarn, has convinced me that they form only chlamydospores somewhat resembling those of *V. nigrescens* and not sclerotia as do both *V. dahliae* and the strains under discussion. He regarded his types as forms of *V. dahliae*, but they certainly are not identical with my new isolates which I propose to call *V. tricorpus* n.sp. (p. 194) because of the three forms of resting body, dark mycelium, microsclerotia and chlamydospores, present. The isolates used in the experiments are as follows:

Designation of isolate	Source of parent culture
V. nubilum (N 1)	Wilted potato stalks from Kent
V. nubilum (N 1 var.)	Hyaline variant of N 1 arising in culture
V. nubilum (N 2)	Soil of strawberry field, Kent; sent to me after being inoculated into and re-isolated from tomato
V. nubilum (N 3)	Centraal Bureau voor Schimmelcultures, Baarn
V. tricorpus (T 1)	Wilted tomato, from Cambridge
V. tricorpus (T 1 var.)	Hyaline variant of T 1 arising in culture
V. tricorpus (T 2)	Wilted tomato, from Fareham, Hants
V. tricorpus (T 3)	Wilted tomato, from Norfolk
V. tricorpus (T 4)	Wilted tomato, from Hertfordshire

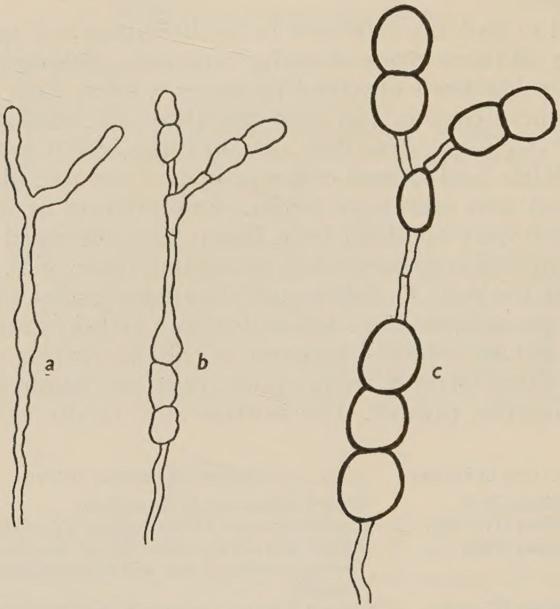
MORPHOLOGY OF ISOLATES

The strains listed were grown from single spores on a wide variety of media in Petri dishes, and on hanging drops of Dox's and potato-dextrose agars. The cultures were exposed to normal daylight at laboratory temperature.

Verticillium nubilum

On potato-dextrose, prune-extract and malt-extract agars respectively, N 1, N 2 and N 3 formed spherical or oval chlamydospores more rapidly and more abundantly than on other media. On potato extract a thin mat of mycelium was formed with very few chlamydospores, while on Dox's, Czapek's and Brown's media hyaline sectors very frequently developed. This was in marked contrast to *V. nigrescens* which formed no such sectors on any media. On both oatmeal and sugar beet-extract agars growth in diameter of the culture and formation of chlamydospores were slower than on other media, but a very thick mat of white woolly aerial mycelium was formed.

Wherever chlamydospores were formed they showed a diameter of 8·5–15·5 μ as compared with the 7–10 μ of *V. nigrescens*, and, whereas the undersurface of the culture of the latter was very dark brown to black, the lower surface of my strains of *V. nubilum* was always black. The under surfaces of the cultures grown in daylight had marked zonations but were uniformly black when grown in darkness.



Text-fig. 1. Development of chlamydospores of *V. nubilum* on a hanging-drop culture of Dox's agar. *a*, after 2–3; *b*, after 4; and *c*, after 7 days' growth respectively. (× 375.)

The sporophores were usually verticillately branched, but frequently single lateral branches bearing conidia were also formed. In hanging-drop cultures in daylight conidia germinated after approximately 18 hr., giving rise to one or two germ-tubes which grew to form uniform hyphae containing oil globules. After 2–3 days the hyphae became very irregular and swollen in places (Text-fig. 1 *a*) with many oil globules concentrated at each swelling. Two days later the mycelium became septate to form large 'cells' (Text-fig. 1 *b*) which were still thin-walled. During the next 3 days these 'cells' increased in size very rapidly and developed thick black walls to form chlamydospores (Text-fig. 1 *c*). Whereas in *V. nigrescens* the mycelium became indistinct between the chlamydospores, in the strains of *V. nubilum* the hyphae remained fairly conspicuous, and sometimes they actually became slightly thickened and blackened.

The hyaline variant grew well on potato-dextrose, Dox's and malt agars, but never formed chlamydospores on any of the media used, neither did the mycelium at any time become swollen and septate. However, in contrast to the hyaline variants of *V. dahliae* and *V. albo-atrum*, which produced conidia only sparingly, that under discussion (N 1 var.) spored profusely.

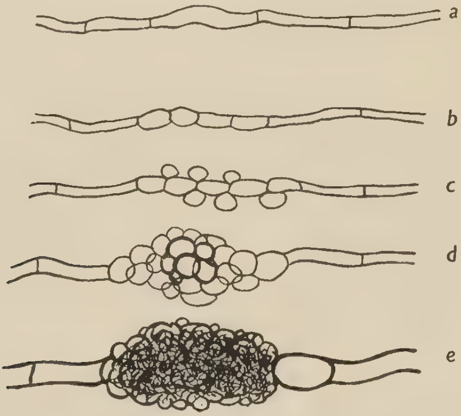
Verticillium tricorpus

On all the media the isolates produced an abundance of verticillately branched sporophores bearing groups of terminal conidia. On prune-extract and potato-extract media the fungi formed rather thin mats of mycelium in contrast to the thick white woolly subaerial mycelium developed on all the others. The best media for development were potato-dextrose, Dox's, and the malt-extract agars. As with *V. nubilnm*, marked zonations of the under surface were observed when T 1, T 2, T 3 and T 4 were grown in normal daylight, as contrasted with the uniformly black under surface when they were grown in darkness.

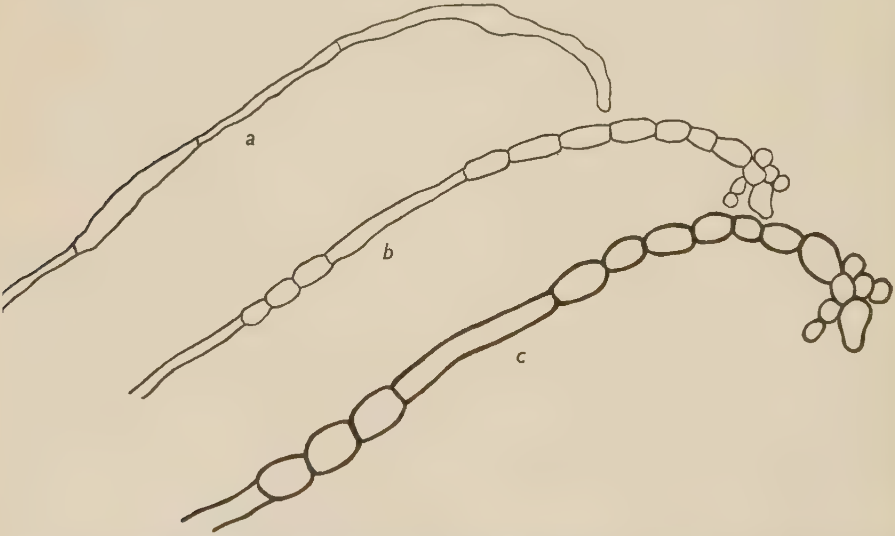
After about 3 days' growth the mycelium in contact with the media, i.e. the prostrate hyphae, developed a conspicuous yellow colour. As the fungus grew over the agar the outer younger hyphae were consistently yellow, but in T 1, T 2, T 3 and T 4 the under surface of the older parts of the colonies gradually became very dark owing to the formation of dark brown to black resting bodies. Where hyaline sectors were formed, and also in cultures of T 1 var. which had not suffered prolonged subculturing, the under surfaces remained yellow. The development of the yellow colour in these T strains was a most characteristic feature, particularly in recent isolates from wilted hosts, and it served to distinguish them macroscopically from cultures of *V. dahliae*, *V. albo-atrum*, *V. nigrescens* and *V. nubilum*, in all of which the young prostrate hyphae were white. However, the intensity of this yellow coloration diminished with prolonged artificial culturing, until the yellow colour became very faint.

On hanging-drops of agar media the spores germinated after about 12 hr. incubation by developing one or two tubes which grew out to form hyphae of uniform width in which oil globules very rapidly appeared. This oil reserve was later seen to be concentrated in the resting bodies. After about 3 days' growth some of the mycelium became septate and increased in diameter to approximately 6–7 μ. Later the walls of these hyphae became thickened and blackened to form dark brown to black resting mycelium (Pl. 10, fig. 1) very similar to that already described and pictured for *V. albo-atrum* (Isaac, 1949).

V. tricorpus, however, differed from *V. albo-atrum* in forming microsclerotia which developed either from one hypha (Text-fig. 2 and Pl. 10, fig. 1), when they remained elongated and narrow (approx. 70 μ in length), or alternatively from a number of hyphae (Pl. 10, fig. 2) when they formed somewhat spherical masses with an average diameter of 80 μ (Pl. 10, fig. 3). Where development occurred from one hypha this became septate (Text-fig. 2 *a*), the 'cells' so formed became swollen (Text-fig. 2 *b*) and then lateral rounded 'cells' were budded off (Text-fig. 2 *c*) to form à compact rather elongated mass (Text-fig. 2 *d*), the walls of which ultimately blackened and thickened (Text-fig. 2 *e*). When several hyphae were involved these became septate, and where they were in contact the 'cells' budded in all planes to form compact almost spherical masses, the walls of the 'cells' in the centre of which began to thicken and blacken, this process extending throughout these bodies, ultimately transforming them into microsclerotia (Pl. 10, fig. 3) which resembled in development and in final structure those of *V. dahliae* (Isaac, 1949).



Text-fig. 2. Development of a microsclerotium of *V. tricorpus* from a single hypha, on a hanging-drop of Dox's agar medium. *a*, after 3; *b*, after 4–5; *c*, after 6–7, *d*, after 9–10; and *e*, after 11–12 days' growth respectively. (× 375.)



Text-fig. 3. Development of chlamydospores of *V. tricorpus* on a hanging-drop of Dox's agar medium. *a*, after 3; *b*, after 5–6; and *c*, after 7–8 days' growth respectively. Note the black resting mycelium lying between the groups of chlamydospores. (× 375.)

The chlamydospores of *V. tricorpus* developed in both intercalary and terminal positions on hyphae which became first septate (Text-fig. 3 *a*) and then swollen, after which they frequently gave rise to lateral sessile buds (Text-fig. 3*b*). All these 'cells' then blackened and thickened to form rectangular to oval chlamydospores (Text-fig. 3*c* and Pl. 10, fig. 1) with average dimensions of 7·5 × 11·0 μ. Those parts of the hyphae which did not form chlamydospores usually became modified to a typical black resting mycelium.

It is therefore clear that, though *V. tricorpus* forms resting structures which are severally identical with similar structures produced by the three species of *Verticillium* described by Isaac (1949), it differs from those other species in the fact that, while each of the latter produced only one type of resting body which is distinctive for each species, each of these isolates of *V. tricorpus* is capable of giving rise to the whole range of such resting structures.

The hyaline variant, T 1 var., grew quite well on all the media with particularly good growth on potato-dextrose, Dox's and Czapek's agar, but in artificial culture it did not recover the ability of the original parent isolate to form black resting bodies, neither did the mycelium become swollen at any time, although septation occurred. T 1 var. resembled the hyaline variant of *V. nubilum* and differed from the variants of *V. dahliae* and *V. albo-atrum* in that it formed abundant conidia.

Dimensions of conidia

Pethybridge reported that the conidia of his *V. nubilum* were usually approximately 9 × 3 μ, whereas in my strains of this fungus the conidia averaged 9·5 × 3·5 μ. *V. tricorpus* formed non-septate rather elongated conidia 4–10 × 2·5–3·5 μ (average 6·5 × 3·0 μ), with rounded ends which contained accumulations of oil globules. They are approximately the same dimensions as those recorded for the spores of *V. albo-atrum*, *V. dahliae* and *V. nigrescens*, but slightly smaller than the conidia of *V. nubilum*.

PHYSIOLOGY OF ISOLATES

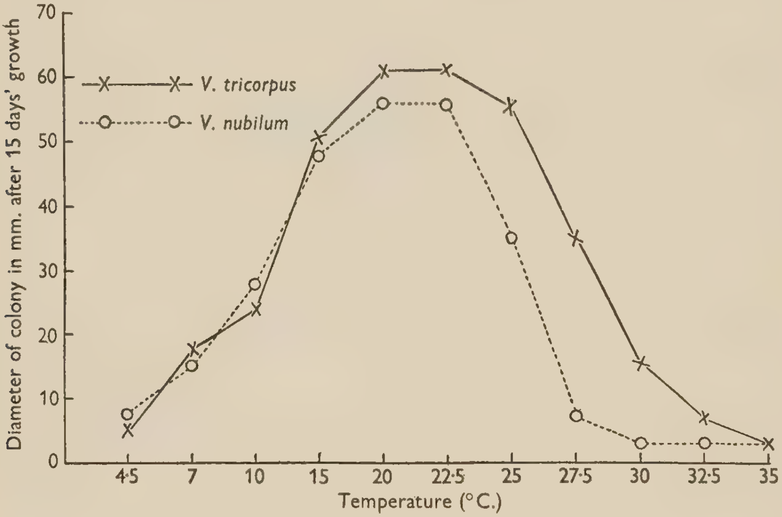
Except where otherwise indicated details of methods used for the present study were similar to those used earlier for *V. albo-atrum*, *V. dahliae* and *V. nigrescens* (Isaac, 1949). Throughout the present investigation *V. albo-atrum*, *V. dahliae* and *V. nigrescens* were grown as controls to ensure close comparison, but in each case the results obtained for these three species were exactly as recorded previously (Isaac, 1949).

Temperature relations

All isolates were grown on three media, viz. normal Dox's, buffered Dox's (normal Dox's buffered at pH 6·4 by sodium and potassium phosphates) and potato-dextrose agars, and all incubated at 4·5, 7·0, 10·0, 15·0, 20·0, 22·5, 25·0, 27·5, 30·0, 32·5 and 35·0° C. respectively. In Text-fig. 4 only the data for N 1 and T 1 on Dox's agar are given since these two strains were typical of their respective groups (except that N 1 var. grew rather faster than the other N isolates), and results obtained for other media were very similar.

Wherever growth occurred the morphology of the cultures was normal, except for *V. tricorpus* at 32·5° C. where the colony assumed an abnormal yeast-like form.

V. nubilum and *V. tricorpus* showed maximum growth at 20·0° C. as well as at 22·5° C., differing from *V. dahliae* which grew best at 22·5° C. and from *V. nigrescens* which developed as well at 25·0° C. as at 22·5° C., but corresponding somewhat closely to *V. albo-atrum*. *V. tricorpus* differed from *V. albo-atrum* and *V. nubilum* and resembled *V. nigrescens* in showing some normal development at 30·0° C., though the growth was not nearly as good as that recorded for *V. dahliae* at this temperature. *V. nubilum* and *V. tricorpus* resembled each other and differed from the three other species in developing well at 15·0° C.

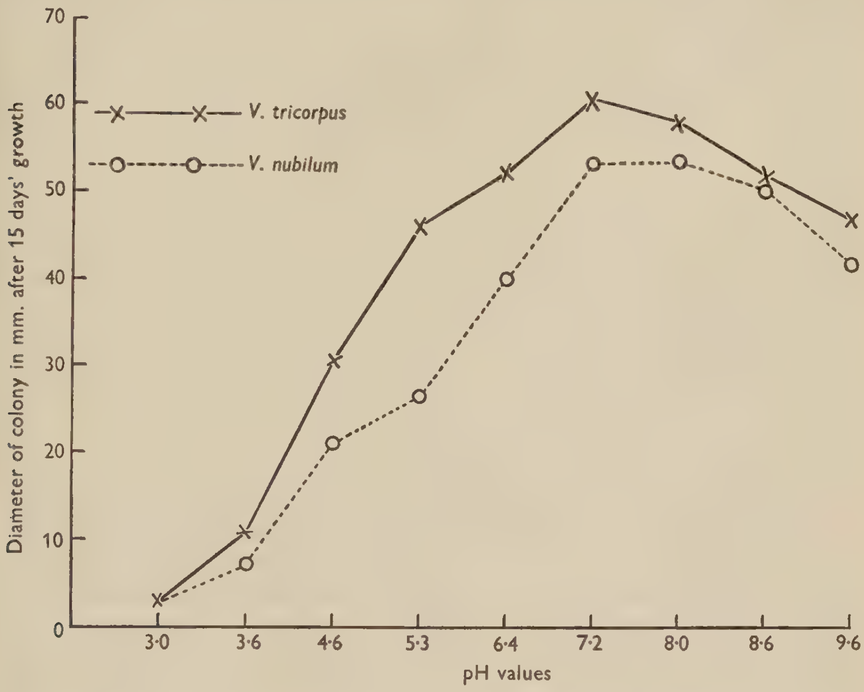


Text-fig. 4. Influence of temperature upon the growth of *V. nubilum* and *V. tricorpus* on Dox's agar medium.

pH of media

All the isolates of *V. nubilum* and *V. tricorpus* and their variants resembled *V. albo-atrum*, *V. dahliae* and *V. nigrescens* in that they rapidly produced a high degree of alkalinity (pH 8·6–9·0) when grown on Dox's (initial pH 5·3) or on potato-extract (initial pH 6·4) agar media, and quickly made a modified Dox's medium (initial pH 5·3) containing 0·2% ammonium sulphate substituted for the usual 0·2 % sodium nitrate, extremely acid (pH 3·2) right to the edge of the plate, although no normal growth had occurred. Where ammonium nitrate replaced the sodium nitrate the strains of *V. nubilum* all grew fairly well (final pH 4·0) as contrasted with *V. tricorpus* which resembled the three other species in showing no normal growth, presumably due to the very acid condition (pH 3·2) produced by these fungi. It would appear that the *V. nubilum* isolates are able to utilize the nitrogen of both the ammonium and the nitrate ions, and show no marked preference for either, in contrast to the other four species which can absorb either ion, but, when both are present, as in ammonium nitrate, show such a preferential absorption of the ammonium ion as to render the medium sufficiently acid to inhibit further growth.

Since potato-dextrose and modified Dox's media (Dox's buffered with sodium and potassium phosphates) remained at a constant pH value during the growth of the isolates of *V. nubilum* and *V. tricorpus*, as had occurred with *V. albo-atrum*, *V. dahliae* and *V. nigrescens* (Isaac, 1949), these media, together with normal Dox's, were adjusted, as described in the earlier paper, to the following pH values: 3·0, 3·6, 4·6, 5·3, 6·4, 7·2, 8·0, 8·6 and 9·6. Three plates of each medium at each pH value were prepared for each isolate and all, after inoculation, were incubated at 22·5° C.



Text-fig. 5. Influence of pH of potato-dextrose agar on the growth of *V. nubilum* and *V. tricorpus*.

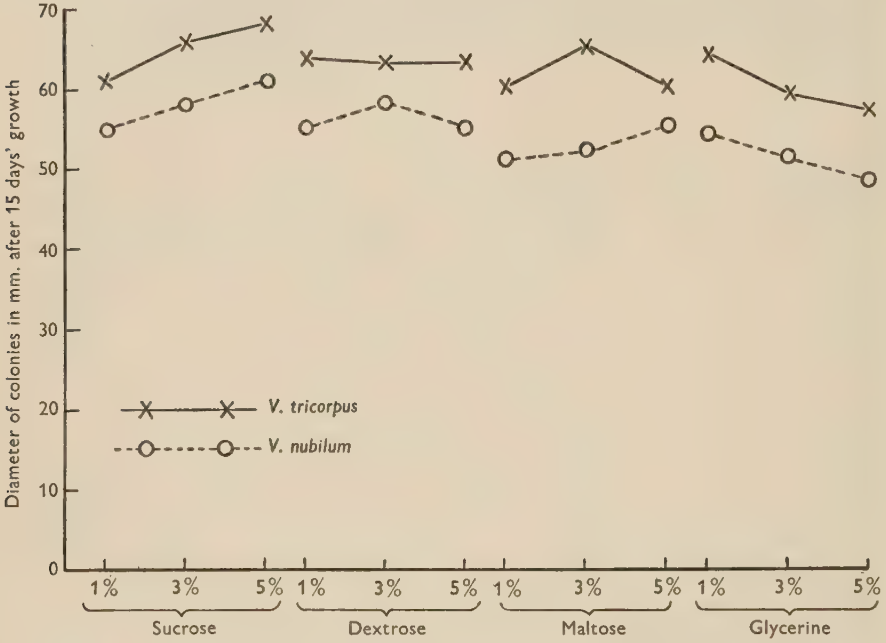
As with the effect of temperature, all the strains in both groups responded similarly to differences in pH on all media, therefore only the results for N 1 and T 1 on potato-dextrose agar medium are shown in Text-fig. 5.

All the strains showed a normal morphology on all media at each pH at which growth occurred except the *V. tricorpus* isolates which at pH 3·6 developed an abnormal yeast-like form.

The strains of *V. nubilum* and *V. tricorpus* showed optimum growths at pH 7·2–8·6 and pH 7·2–8·0 respectively, and both therefore differ from *V. dahliae* and *V. nigrescens* (optimum at pH 5·3–7·2), and *V. albo-atrum* (optimum at pH 8·0–8·6) (Isaac, 1949), while they resembled the last-named species in developing very poorly at pH 3·6, and conformed to the reaction of all the others in failing to grow at pH 3·0.

Variation in the source of carbon

The same sources of carbon and the same percentage of each were employed for these experiments as were used previously (Isaac, 1949), viz. 1·0, 3·0 and 5·0 % of sucrose, dextrose, maltose and glycerine respectively, replacing the normal 1·5 % sucrose in Dox's medium. As before, three replicates of each of these twelve media were prepared for each isolate and all the plates, after inoculation, were incubated at 22·5° C.



Text-fig. 6. Influence of various carbon compounds upon the growth of *V. nubilum* and *V. tricorpus*

Apart from the fact that N 1 var. grew consistently more rapidly and T 1 var., in general, more slowly than their respective parent types, all the isolates within each group reacted similarly, so only the data for N 1 and T 1 are shown in Text-fig. 6.

In general, all the fungi on all media grew true to type. The amount of resting bodies and of white aerial mycelium increased with an increase in the percentage of each carbon source. Sporulation on all media was normal.

Although *V. nubilum* developed much faster than did *V. nigrescens*, they were similar in showing maximum growth with dextrose and sucrose, as contrasted with *V. albo-atrum* which developed poorly on these media. *V. nigrescens*, unlike *V. nubilum*, produced chlamydospores equally abundantly on every medium irrespective of the percentage of specific nutrient included. *V. tricorpus* resembled *V. dahliae* to some extent, and differed from the other three species in developing well on all media, although even with *V. dahliae* growth was markedly arrested when the concentration of glycerine was increased (Isaac, 1949). Thus in their response to various sources of nutrient carbon *V. nubilum* and *V. tricorpus* show physiological differences from all the other species as well as from each other.

Variation in the source of nitrogen

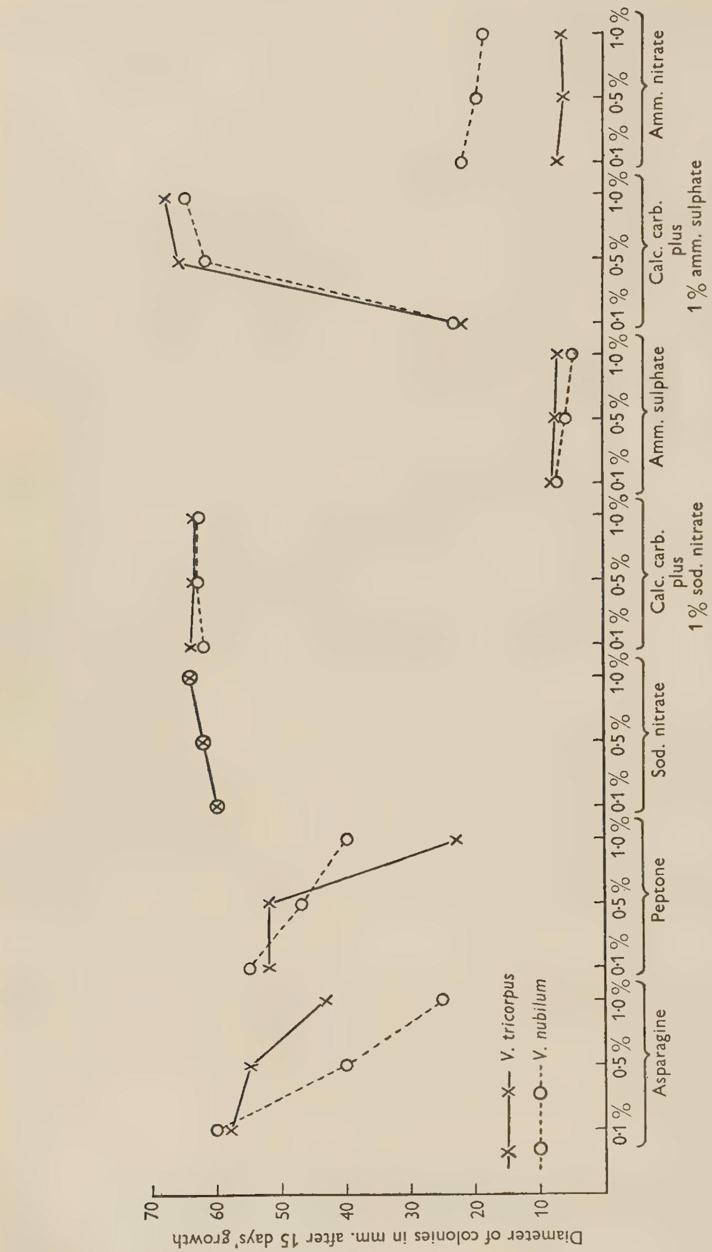
As in previous experiments (Isaac, 1949) all the isolates of *V. nubilum* and *V. tricorpus* were grown on modified Dox's media containing, as a substitute for the normal 0·2 % sodium nitrate, 0·1, 0·5 and 1·0 % respectively, of asparagine, peptone, sodium nitrate, ammonium sulphate and ammonium nitrate. They were also grown on 1·0 % ammonium sulphate medium buffered with calcium carbonate (0·1, 0·5 and 1·0 % respectively) and on 1·0 % sodium nitrate medium similarly buffered. Three plates of each of these twenty-one media were used for inoculation by each strain, and all were incubated at 22·5° C. As all the isolates in each group gave very similar responses, only data for N 1 and T 1 are shown in Text-fig. 7.

Asparagine. On 0·1 % asparagine all the colonies showed good normal growth; thick mats of aerial woolly mycelium developed; resting bodies were formed abundantly and sporing was heavy. With an increase in concentration the fungi showed 'staling' effects which became very marked on 1·0 %. Here development was poor; the margins of the colonies budded in a yeast-like manner; conspicuous radiating cracks were present; resting bodies were only sparsely developed, although under the microscope the hyphae of N 1, N 2 and N 3 and T 1, T 2, T 3 and T 4 appeared septate, the 'cells' being slightly swollen but rarely thickened or blackened; the aerial mycelium of *V. nubilum* appeared white while that of *V. tricorpus* was pale brown; sporing was light on all cultures.

Peptone. V. tricorpus on all the concentrations, and *V. nubilum* on the lower concentrations, grew true to type. On 1·0 % peptone growth of *V. tricorpus* was very slow, and that of *V. nubilum* was poorly developed with conspicuous ridges and cracks radiating from the centre, conidia were only sparingly formed, and only few chlamydospores were produced by N 1, N 2 and N 3.

Sodium nitrate. On the media containing sodium nitrate growth of all isolates was normal, but there was a marked tendency for hyaline sectors to form. Increasing the concentration of this salt resulted in a more marked yellowing of the prostrate hyphae of *V. tricorpus*, while addition of calcium carbonate had no apparent effect on growth of any isolate on these media.

Ammonium sulphate. On unbuffered ammonium sulphate media all the isolates appeared as small abnormal yeast-like masses with neither conidia nor black resting bodies, yet these colonies when transferred to the usual Dox's medium grew to form normal cultures. Where 0·1 % calcium carbonate was added growth was just a little better, but no resting bodies formed and sporing was very light. With the addition of both 0·5 and 1·0 % calcium carbonate all the fungi developed excellently and true to type.



Text-fig. 7. Influence of various sources of nitrogen upon the growth of *V. nubilum* and *V. tricorpus*

Ammonium nitrate. On unbuffered ammonium nitrate media *V. tricorpus* appeared as on ammonium sulphate media. *V. nubilum* grew slowly, mycelium and conidia developed normally, but very few chlamydospores were formed.

All the isolates grew best on the strongly buffered ammonium sulphate medium, thus resembling *V. albo-atrum*, *V. dahliae* and *V. nigrescens* (Isaac, 1949). On the asparagine media *V. nubilum* and *V. tricorpus* and the above three species again showed similar reactions, viz. increasing the concentration of asparagine slowed down growth, although this was only slight in *V. nigrescens*. *V. tricorpus* resembled *V. nubilum*, in a marked slowing down of development with increasing concentration of peptone, in contrast to the other three species in which such increase accelerated growth. Variation in the concentration of sodium nitrate had no appreciable effect on *V. nubilum* and *V. tricorpus* which in this respect resembled *V. albo-atrum* but differed from *V. dahliae* and *V. nigrescens* (in which an acceleration of growth occurred with increasing concentration). On the ammonium nitrate medium *V. tricorpus*, like *V. albo-atrum*, *V. dahliae* and *V. nigrescens*, showed very little growth, coincident with a rapid accumulation of acid; *V. nubilum*, however, did show some development on this medium. Significant physiological differences between all these species of *Verticillium* are thus indicated by their varying responses to a supply of nutrient nitrogen.

STUDIES IN PATHOGENICITY

Pethybridge (1919) carried out a number of inoculation tests on living potato stalks and tubers with his *V. nubilum* and *V. nigrescens* but no infection occurred. I have shown (Isaac, 1949) that *V. albo-atrum* and *V. dahliae* are pathogenic to sainfoin (common), antirrhinum, cucumber, tomato (Kondine Red), egg-plant, strawberry (Royal Sovereign, Malling 40), potato (Eclipse), chrysanthemum, hop (Fuggle) and salsify, while *V. nigrescens* is pathogenic to all these except sainfoin, strawberry and cucumber. Inoculation experiments were now conducted in order to compare *V. nubilum* and *V. tricorpus* with the other three species in their effects on all these eleven hosts with the addition of lupin, tobacco and carnation.

Wound inoculations of series of six plants of each host were made by inserting mycelium of each of the isolates of *V. nubilum* and *V. tricorpus* into wounds made in the xylem just below ground level. Recent re-isolates of the fungi were used to ensure that no possible loss of pathogenicity might result from prolonged artificial culturing. Plants of each host with wounds only, and three others of each host inoculated with each of the other three species of *Verticillium* were kept as controls for all the experiments.

Soil inoculation experiments with sainfoin, potato, tomato and antirrhinum were carried out by adding 4-week-old cultures of the respective fungi on sterilized wheat grains to soil in pots in which the seedlings were then planted. Control plants in soil containing uninfected wheat grains and series of three specimens of each host in soil infected with each of *V. dahliae*, *V. albo-atrum* and *V. nigrescens* respectively were also kept. The experiments were conducted in the summer months, the plants in pots being kept in cold frames or in the open.

These wound and soil inoculation investigations showed that *V. nubilum* was pathogenic to tomato and potato only, while *V. tricorpus* was pathogenic to tomato only. Results, cited above, obtained previously for the other three species of *Verticillium* were corroborated, and it was also shown that *V. dahliae* and *V. albo-atrum* were pathogenic to lupin and tobacco but not to carnation, whereas *V. nigrescens* failed to induce wilt in any of these three hosts. *V. nubilum* in potato and tomato and *V. tricorpus* in tomato produced wilt symptoms 6–8 weeks after wound inoculation, while in the soil series 8–10 weeks elapsed between inoculation and the appearance of the disease, as contrasted with *V. albo-atrum*, *V. dahliae* and *V. nigrescens*, which induced wilt much more rapidly in these hosts. Since *V. nubilum* and *V. tricorpus* are pathogenic to a narrow range of host plants, and since the external symptoms of disease are induced only after a protracted time, it appears evident that these isolates are weaker pathogens than any of the other three species under consideration.

All the fungi produced similar symptoms of wilt—at first the lower leaves showed loss of green pigmentation, became desiccated then fell; later the higher leaves became similarly affected until the whole plant was defoliated. The wood of the diseased plants was discoloured by gum and mycelium in the vessels and by the brown staining of the walls of these elements. The mycelium present in the xylem vessels stimulated the production of tyloses—a typical reaction of many hosts to parasitism by species of *Verticillium*.

The causal organism of the disease was obtained from all the wilted plants and was identical with the inoculum except that where T 1 var. was re-isolated it produced the three types of black resting bodies (i.e. it had reverted to type).

Although wilt symptoms were induced neither in antirrhinum inoculated with *V. nubilum*, nor in antirrhinum, tobacco and lupin with *V. tricorpus*, both species were re-isolated from the respective wounded plants 5–6 months after inoculation, and the re-isolate was always identical with the inoculum with the exception of T 1 var. which had reverted to type. Generally the mycelium had moved into the xylem vessels to a distance of approximately 1 in. above and half an inch below the wound, but there were no gum deposits nor discoloration of the walls of these elements. Where antirrhinum was grown in inoculated soil, N 1, N 2, T 1 and T 3 were re-isolated from some plants, but again the mycelium was restricted to small areas in the roots, suggesting that although infection had probably occurred through small wounds the parasites had been unable to extend appreciably beyond the region of original infection. Keyworth (1947) reported this phenomenon in certain resistant strains of hop in which *V. albo-atrum* was present in the vascular system but caused no external symptoms of wilt.

These studies in pathogenicity again demonstrate very wide differences between the two groups of isolates under discussion and the other three established species of *Verticillium*.

CONCLUSIONS AND DISCUSSION

Evidence has already been produced (Isaac, 1949) justifying the separation of *V. dahliae*, *V. albo-atrum* and *V. nigrescens* as distinct species on morphological, physiological and phytopathological grounds. Throughout the present work evidence has also accumulated to the effect that though *V. nubilum* and *V. tricorpus* possess certain features indicating affinities with each other and with the other three species, nevertheless major differences are apparent between them all. The chief characteristics of all these forms are compared in Table 1.

Table 1. *Comparison of species of* Verticillium

	V. dahliae	*V. albo-atrum*	*V. nigrescens*	*V. nubilum*	*V. tricorpus*
Resting bodies	Microsclerotia	Resting mycelium	Chlamydospores (diam. 7–10μ)	Chlamydospores (diam. 8·5–15·5μ)	Microsclerotia, resting mycelium and chlamydospores (diam. 7·5–11·0μ)
ur of young strate hyphae	White	White	White	White	Yellow
line sectors	Very frequent	Frequent	Never recorded	Very frequent	Very frequent
imum temperature growth (° C.)	22·5	20·0–22·5	22·5–25·0	20·0–22·5	20·0–22·5
wth in culture 30° C.	Fairly good	Nil	Fairly good	Nil	Only fair
imum pH for wth	5·3–7·2	8·0–8·6	5·3–7·2	7·2–8·6	7·2–8·0
wth at pH 3·6	Fairly good	Fair	Good	Very poor	Yeast-like mass
wth in ammonium rate medium	Nil	Nil	Nil	Fairly good	Nil
source of carbon	Sucrose and dextrose	Glycerine	Sucrose and dextrose	Sucrose and dextrose	Sucrose, dextrose, maltose and glycerine
rease in concentra- n of peptone	Stimulates growth	Stimulates growth	Stimulates growth	Slows down growth	Slows down growth
t range	Wide	Wide	Fairly wide	Tomato and potato only	Tomato only
ction of host	Rapid	Usually very rapid	Fairly rapid	Slow	Slow

The formation of chlamydospores by *V. nubilum*, which are much larger than those of *V. nigrescens*, and the consistent simultaneous development of microsclerotia, resting mycelium and chlamydospores and the yellow coloration of young prostrate hyphae in *V. tricorpus* are certainly distinctive features, which, together with the other differences listed above, sharply differentiate these isolates from each other and from *V. albo-atrum*, *V. dahliae* and *V. nigrescens*.

So long as sexual reproduction in the genus is unknown the question as to whether the morphological, physiological and phytopathological differences which have been enumerated entitle any of these members of the genus to specific, varietal or form rank must very largely remain one of personal preference. When dealing with *V. albo-atrum*, *V. dahliae* and

V. nigrescens (Isaac, 1949), I accepted the principle suggested by Butler (1926) that convenience (usefulness, simplicity and practicability) is the ultimate criterion of classification, and argued that specific rank should be ascribed to these three species. I must therefore refuse to 'lump' the N and T isolates into one group-species. Since the inherent differences between these isolates and each of the three known species are at least as great and fundamental as are their differences from one another, there is, in my view, every justification for according specific status to these two groups of *Verticillium* isolates.

A comparison of the microphotographs of the chlamydospores of Pethybridge's *V. nubilum* (Pl. 10, fig. 4) with those of my N strains (Pl. 10, fig. 5) shows that the resting structures of these organisms are similar. My isolates were pathogenic to Kondine Red tomato and Eclipse potato, whereas *V. nubilum* was originally described by Pethybridge as saprophytic only; but, since there is no record of any tests of his fungus on this variety of potato, and since he did not report any inoculation experiments on tomato, this difference does not appear to be sufficient to warrant a subdivision of his species. It is proposed therefore to ascribe to my N strains the name *Verticillium nubilum* Pethybr.

Since the T strains have not been described previously and since they form the three types of resting structures, I propose to name them *Verticillium tricorpus* sp.nov.

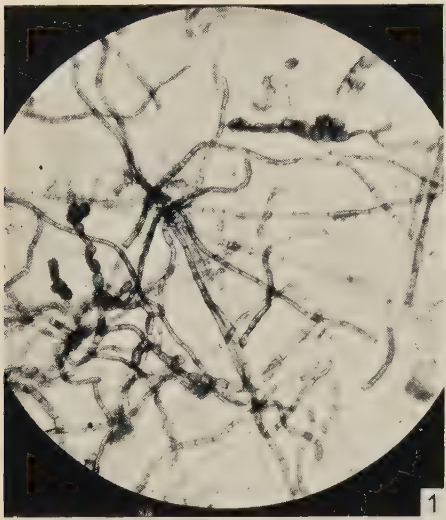
Verticillium tricorpus sp.nov.

Mycelium erectum album septatum; mycelium novissimum prostratum colore croceo; 2–3 μ app. crassum cum globulis olei conspicuis. Hyphae fertiles subrectae, verticellate ramosae cum apicali conidiorum globo, hyalinorum et unicellularium, quadratorum vel oviformium, 4·0–10·0 longorum × 2·5–3·5 μ crassorum, av. 6·5 × 3·0 μ. Mycelium nigrum quiescens, app 6–7 μ crassum; microsclerotia av. 80 μ diam.; chlamydosporae nigrae, av. 7·5 × 11·0 μ. Hab. Lycopersicum esculentum marcescens. Prope Fareham, Hants., Anglia, 1950.

Type material of this new species will be deposited in the Herbarium of the Commonwealth Mycological Institute, Kew; in the Herbarium, Royal Botanic Gardens, Kew; and in the Centraalbureau voor Schimmelcultures, Baarn, Holland.

The fact that each of the other four species is capable of developing one, and only one, type of resting body, while the new species may develop all types simultaneously implies, to my mind, inherent genic differences between all five species; but whether *V. tricorpus* is more primitive and has given rise to the other forms by changes in the genic characters determining the formation of the resting structures, or whether it is a derivative form which has acquired new powers of reproduction during the course of its evolution remains an interesting but at the moment unsolved problem.

My thanks are due to Prof. F. A. Mockeridge for her advice and criticism in preparing the paper, to Mr W. R. Smyth for help in preparing the Latin diagnosis and to Dr R. E. Taylor and the East Malling Research Station for providing me with specimens of the fungi.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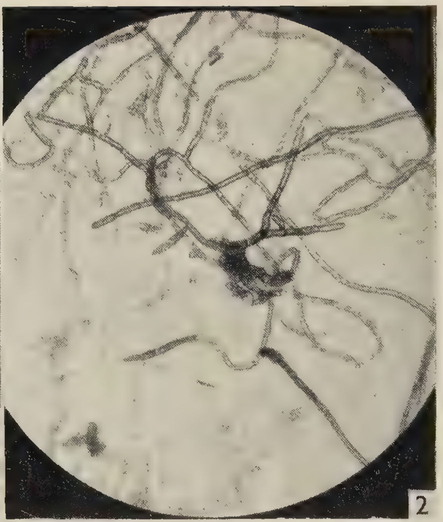
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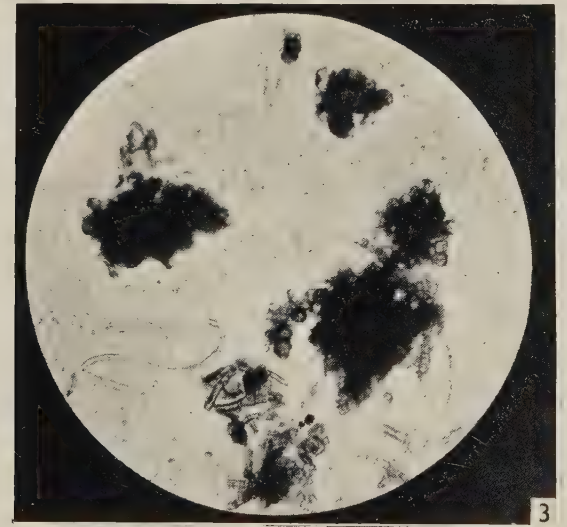
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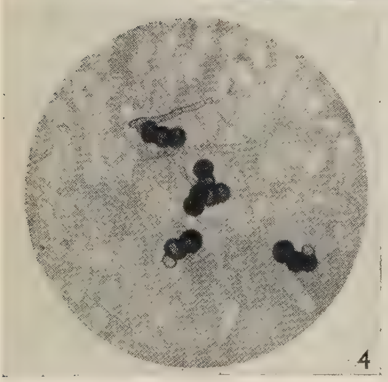
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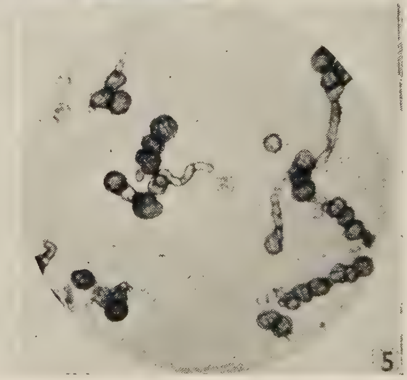
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EXPLANATION OF PLATE 10

Fig. 1. Resting mycelium, chlamydospores and a long narrow microsclerotium developed from a single hypha of *V. tricorpus* on Dox's agar medium. (× 200.)

Fig. 2. Resting mycelium and a small microsclerotium developed from a number of hyphae of *V. tricorpus* on Dox's agar medium. (× 200.)

Fig. 3. Typical microsclerotia of *V. tricorpus* on Dox's agar medium. (× 200.)

Fig. 4. Reproduction of microphotograph of chlamydospores of *V. nigrescens* Pethybr. (× 315.)

Fig. 5. Chlamydospores of the *V. nubilum* isolates discussed in this paper from a pure culture on Dox's agar medium. (× 300.)

(*Accepted for publication 4 April* 1952)

SCHIZONELLA MELANOGRAMMA: A NEW RECORD FOR BRITAIN

By E. W. BUXTON

Botany School, University of Cambridge

(With 1 Text-figure)

On 5 May 1952, specimens of *Carex ericetorum* were collected at Foxhole Heath near Icklingham, Suffolk; the leaves of these plants bore several black elongate sori of a smut fungus. Microscopic examination showed this to be *Schizonella melanogramma* (DC.) Schroeter, a member of the Ustilaginales hitherto not recorded for Britain. This fungus is known on various Carices in Europe (Ciferri, 1938) and in America (Clinton, 1905) on the leaves of *Carex pennsylvanica*. The first description is that of De Candolle (1815) under the name *Uredo melanogramma*, the specimens being taken from *Carex montana* and *C. digitata* from the Jura mountains.

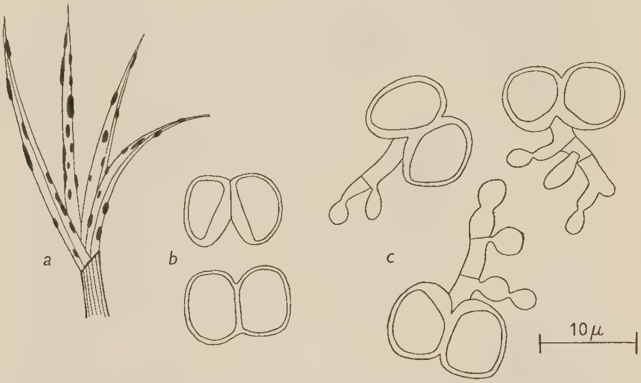

Fig. 1. *Schizonella melanogramma. a*, infected plant of *Carex ericetorum* showing elongate black sori, × 1; *b*, paired spores; *c*, spores germinating in hanging water-drop. Basidia bearing basidiospores which bud profusely in a yeast-like manner.

The genus *Schizonella* was erected by Schroeter (1877). Ciferri (1938) accepted the genus and gave a Latin diagnosis of material of *S. melanogramma* on *Carex montana*. My specimens agree with this description and have also been checked with the collection of specimens at Kew which includes material under the name *Schizonella melanogramma* collected by Clinton in Ohio, 1900, Wroblewski in Poland, 1927, and Winter in the Tyrol, 1893. The reddish brown spores are produced in black elongate sori which occur on both sides of the leaf and vary in length from 1 mm. to about 8 mm. The spores measure 7–10 μ × 9–13 μ and are joined in pairs by a very short hyaline canal.

An account of *S. pusilla* (Cooke & Peck) Ciferri is given by Ciferri (1931). This species differs from *S. melanogramma* in its spore measurements on various *Carex* host plants, but Ciferri reserves his judgement on the validity of separating the two species until actual biological differences are found.

On germination of the spores, which occurs readily in water or on plain agar, a short promycelium is produced from between the two parts of the spore-pair. The four basidiospores bud profusely in the manner of yeast. The sori, according to Schellenberg (1872), are found in early spring and the fungus overwinters in the rootstock of the host plant. Nothing appears in the literature concerning the mode of infection and the full life cycle; the host plants, at least *C. ericetorum* and *C. ornithopoda*, are rare and local in Britain.

Whilst the identification was in progress, Dr A. S. Watt, of the Botany School, Cambridge, informed me that he had collected diseased material of *C. ericetorum* at the same locality in the Breckland in July 1940. This had been tentatively identified by Dr W. J. Dowson as *Schizonella melanogramma*, but no herbarium specimens had been kept.

Specimens of *Carex flacca* collected by Mr N. F. Robertson on the Coyles of Muick, Ballater, Scotland, in June 1951 proved to bear sori of the same fungus. Specimens from Suffolk and from Ballater have been deposited in the Mycological Herbarium at Cambridge under index numbers 14 and 15. Material from these collections has been sent to Kew Herbarium and to the Commonwealth Mycological Institute.

My thanks are due to the Director of Kew Gardens and to Dr R. W. G. Dennis of Kew Herbarium for making available the collection of *Schizonella* specimens and for the use of the library.

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CROWN ROT, A SEEDLING DISEASE OF GROUNDNUTS CAUSED BY *ASPERGILLUS NIGER*

By I. A. S. GIBSON

Overseas Food Corporation

(With Plate 11 and 1 Text-figure)

Inoculation experiments have established *Aspergillus niger* van Tieghem as the cause of Crown Rot of groundnut seedlings in East Africa. Typical lesions were produced by mycelial inocula on a suitable substrate, but no infection could be obtained by the use of conidia alone. The pathogenicity of five isolates of the *A. niger* group (Thom & Raper, 1951) could be closely correlated with oxalic acid production, but not with that of citric acid. Hypocotyl lesions were produced by treatment with growth products of pathogenic isolates in agar, and also by treatment with pure oxalic acid in comparable concentration.

The optimum temperature for growth of *A. niger* on agar was found to be 37° C.; seedlings raised at 30–37° C. were most disposed to severe development of Crown Rot, and the progress of the disease was most rapid at these temperatures. Under experimental conditions, development of Crown Rot was most severe in a soil of light texture.

Introduction

The field observations on which this paper is based were made in Tanganyika, from 1949 to 1951, while the author was holding the post of Regional Plant Pathologist to the Overseas Food Corporation at Urambo. Complementary laboratory experiments were carried out subsequently at Cambridge. The first records of this disease in Tanganyika were made by Wallace (1948) and the work described below followed his original identification of the disease as Crown Rot. The name Crown Rot is taken from a description of a similar condition of groundnuts by Morwood (1946) from Queensland.

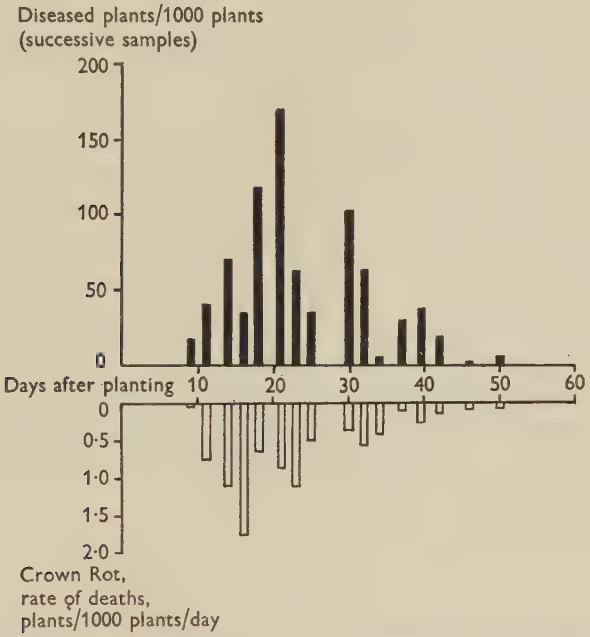
Earlier reports of Crown Rot were made by Jochems (1926), who first described the disease and carried out inoculation experiments (which will be discussed later) using *Aspergillus niger*, and by Morwood (1945, 1946) and Blackie (1947), who have given accounts of similar seedling diseases from Queensland and Papua, respectively. The observations of these workers agree in essentials with those made in East Africa. Crown Rot is also known in South Africa, though no published report of its occurrence there has been found.

Field symptoms and distribution of the disease

Crown Rot is characterized in the field by the wilting and death of seedling groundnuts accompanied by a rotting of the hypocotyl. This hypocotyl rot is characterized by a yellow-brown lesion extending into the tissue with a profusely sporulating growth of *A. niger* van Tiegh.;

eventually the hypocotyl may become blackened and rotted throughout (Pl. 11, fig. 1).

In East Africa the onset of wilting is so rapid that it was necessary to record and remove diseased plants at weekly intervals from observation plots. If this were not done, there was a risk of dead plants being lost through wind or rain-wash. It has also been observed that seedlings may suffer from slight but definite Crown-Rot lesions, but remain otherwise healthy. Text-fig. 1 gives the results of a field experiment which was carried out to investigate the relationship between incidence of the lesions and that of deaths due to Crown Rot, on a section of a normal production field. Observations on the death-rate of plants due to Crown Rot were made at intervals of 2 or 3 days over the whole of this section, all dead plants being removed at each inspection. The frequency of hypocotyl infections was determined simultaneously by digging up three or four row lengths and recording total numbers of seedlings and numbers of hypocotyl lesions. When the death-rate from Crown Rot had reached a low level (at about the 50th day after planting) the final count for plant deaths was made and the residual plants throughout the section were removed, counted, and recorded for lesions.



Text-fig. 1. Variation of Crown-Rot incidence with plant age.

From these records it can be seen that there was first a rise and then a fall in the frequency of plants with hypocotyl lesions in successive samples, which corresponds approximately with the curve for death-rate. The peak for death-rate occurred at about the 17th day (as generally observed elsewhere) whereas that for hypocotyl lesions occurred somewhat later, at about the 22nd day. From these data it appears that many lightly infected plants must eventually have recovered, as the fall in numbers of such plants at later samplings cannot be accounted for by the death of seedlings; thus between 20 and 32 days after germination, the disease rate fell from 170 to 64/1000 plants, whereas total deaths over the same period were only 8/1000 plants. The period over which this fall in infection takes place coincides with a change in the hypocotyl structure in which the fleshy cortex tends to collapse and the development of a more woody tissue ensues. A full account of this process is given by Yarbrough (1949). During this period it is possible that many light infections may become indetectable by visual inspection or may be sloughed off entirely.

In some cases where severe lesions have occurred on the lower part of the hypocotyl under conditions of high soil moisture, adventitious rootlets have arisen above the seat of infection and enabled the plant to survive until the surface soil dried out once more (Pl. 11, fig. 1). These cases were rare. The disease showed no tendency to spread from plant to plant along rows, and there was also no tendency for the disease to increase in fields cropped to groundnuts over two or three successive years.

Finally, it is important to note that in East Africa the disease was widespread only in the large-scale cultivations of the O.F.C., and here the incidence was generally low. In native groundnut fields, it was never recorded. This may be due to the very different histories of these soils, and also to the general practice of seed treatment in the O.F.C. which is found to increase the incidence of Crown Rot (Gibson, 1953). Furthermore, cultivations of the O.F.C. were never of a mixed nature, and the soil was subject to greater drying-out than that of native fields, which were frequently under a mixed crop of cereal and groundnut, and sometimes also had a high weed population. The conditions in the O.F.C. would thus lead to a greater predisposition to wilting in seedlings where light Crown-Rot infections occurred.

Etiology of Crown Rot

Isolations made from infected tissues most commonly yielded the following fungi: *Fusarium oxysporum* sensu Snyder & Hansen, *F. equiseti* (Corda) Sacc., *F. solani* sensu Snyder & Hansen, *F. culmorum* (W. G. Smith) Sacc., *Rhizopus arrhizus* Fischer, and *Aspergillus niger* van Tiegh. Of these *A. niger* was consistently found, whereas the *Fusarium* spp. were common, but not always present. Following these observations, surface sterilized hypocotyls of seedling groundnuts (variety Natal Common) were inoculated with *A. niger* from infected plants, and then incubated under damp conditions in sterile containers. Methods of inoculation and results are given in Table 1.

The infections resulting from these inoculations closely resembled those of Crown Rot observed in the field, and *A. niger* was re-isolated from the infected tissues. Uninoculated controls remained healthy. No other fungi were obtained in the course of re-isolations, and sections cut through diseased tissue revealed the presence of fungal hyphae only. These observations indicate that the causal agent of this disease is *A. niger*. Although in these experiments no infection of the unwounded hypocotyl was obtained from conidia in a water suspension, Jochems (1926) has claimed to have obtained infection by the application of spore suspensions around plants growing in soil. I have tried this method on plants growing in sand under glasshouse conditions in Cambridge, but without success. Possibly Jochems's results were due to the initial infection of cotyledons below soil level leading, as in many natural infections, to a hypocotyl invasion. This did not occur in my experiments as the cotyledons were mainly above the soil surface. It seems likely, though no test has been made, that the cotyledons, unlike the hypocotyl, are susceptible to infection by conidia alone. It is also possible that, if Jochems had used sterilized soil for his inoculation experiment, *A. niger* would have established itself in mycelial culture throughout the soil. Field observations in East Africa have suggested that natural infection occurs chiefly in the following three ways:

Table 1. *Etiology of Crown Rot: summary of inoculation experiments with* Aspergillus niger

Inoculum	Method of inoculation	No. of seedlings with Crown-Rot symptoms/No. of seedlings inoculated
Conidia in water	By brush to surface of hypocotyl	0*/30
Conidia in water	By needle scratch into hypocotyl tissue	12/93
Conidia and mycelium	0·5 cm. squares of mycelial mat placed on hypocotyl	8/10
Conidia and mycelium	0·5 cm. squares of an agar culture placed on hypocotyl	45/54

* Some plants were observed with a slight superficial growth of mycelium but no lesions.

(i) By contact between a previously infected cotyledon and the hypocotyl in the course of emergence.

(ii) By contact of the hypocotyl with *A. niger* established in organic debris in the soil.

(iii) In slightly older seedlings, through previously infected cotyledons dropping off and leaving bases from which infection may spread to give a true collar rot.

It has not been practicable to assess the frequency with which each type of infection occurs in nature.

HOST-PARASITE RELATIONSHIPS IN CROWN ROT

During the work in East Africa, two morphologically distinct isolates of *A. niger* (later referred to as Isolates 7 and 29) were obtained from infected tissues. Inoculation experiments showed that both were pathogenic. Later experiments were made at Cambridge to compare the pathogenicity of these isolates with that of three from other sources. Details of the five isolates are given below.

Isolate 7. From Crown-Rot-infected tissues in East Africa, differed from Isolate 29 in having a higher growth rate and a greater development of aerial mycelium in culture, and has been identified as *A. niger*.

Isolate 15. Obtained as a sector in a culture originating from infected tissue. The original culture was very similar to Isolate 29; the sector was characterized by a lower growth rate, absence of aerial mycelium and formation of a pronounced yellow pigment, and was subsequently identified as *A. foetidus* Thom & Raper.

Isolate 28. Maintained in the culture collection of the Cambridge Botany School since 1928 and identified as *A. awamori* Nakazawa.

Isolate 29. Obtained from Crown-Rot-infected tissue in East Africa, and subsequently identified as *A. niger*.

Isolate 34. Isolated by Mr E. Hainsworth from infected tea flowers in Assam, and subsequently identified as *A. violaceo-fuscus* Gasp.

All five isolates fall within the *A. niger* group of Thom & Raper (1951).

Before the inoculation experiments were carried out, assessments were made of the growth rates and acid production of the five isolates. These were carried out in growth-rate tubes similar to those used by Ryan, Beadle & Tatum (1943), on a 2 % dextrose-Dox medium to which bromocresol green had been added. Growth rates were measured after 96 hr. at 25° C., and at the same time an estimate of acid production was made by measuring the zone width of colour change of the indicator beyond the edge of the colony. This method of determining acid production is only very approximate, as it depends on the type of acid produced as well as the quantity. Results of these determinations are given in Table 2.

Table 2. *Pathogenicity and acid production of five isolates of the* Aspergillus niger *group*

Isolate no.	Acid producing capacity (cm.) (indicator)	Growth rate on agar (cm./24 hr.)	Pathogenicity ratings	
			At 30° C.	In glasshouse
15	4·3	0·20	92	24
29	3·3	0·33	81	23
7	3·3	0·48	61	19
28	Nil	0·40	28	7
34	Slight	0·68	5	3
Control	.	.	2	0

Two inoculation experiments were carried out. At a suitable time after seedling germination, the soil was excavated from around the hypocotyl which was then inoculated with an agar disk cut with a cork-borer (8·5 mm. diam.) from a growing colony of *A. niger*; after inoculation, the soil was replaced. Control seedlings were similarly treated but received disks of sterile agar only. In the first experiment, seedlings were grown in sand culture at a constant temperature of 30° C. and under artificial light; these plants tended to be etiolated but were otherwise healthy. In the second experiment seedlings were grown in the glasshouse in a 1 : 3 soil/sand mixture under fluctuating temperature and normal daylight conditions; these plants were normal but grew rather slowly owing to the relatively low temperature. Scores were made in the first experiment when the disease was well advanced, 8 days after inoculation. In the second experiment the progress of the disease was much slower and a final assessment was made 13 days after inoculation. Disease assessments in these experiments, and in all other experiments to be reported below, were made as follows.

Score 5. Plants dead and suffering from typical hypocotyl rot.

Score 4. Plants wilting slightly, with extensive hypocotyl rot.

Score 2. Plants with pronounced yellow lesions on the hypocotyl, but with little fungal growth.

Score 0·5. Plants with slight yellow lesions on the hypocotyl.

For a single treatment each plant was scored individually and the total score for the treatment was stated as a percentage of the maximum possible score (number of plants in treatment × 5).

From the results of these two experiments (Table 2) there is clearly a considerable range in pathogenicity of the isolates studied; secondly, there appears to be a close correlation between acid production by the respective isolates and their pathogenicity to groundnut seedlings.

In view of these results, further experiments were carried out to determine the acid-producing capacity of these isolates more exactly, and to obtain information on the nature of the acids formed. For these experiments cultures of isolates were grown on a liquid 2 % dextrose-Dox medium in 100 ml. flasks at 25° C. for a period of 140 hr. After this period, the media were filtered off from the mycelium by Seitz filter and kept at freezing-point until required for further examination. These culture filtrates were tested qualitatively for organic acids common as metabolic products of *A. niger* by a chromatographic method. The result of this showed that oxalic acid was produced in appreciable quantities by the pathogenic Isolates 7, 15 and 29, and in smaller quantities by Isolate 28.

Table 3. *Acid production by five isolates of the* Aspergillus niger *group*

	Isolates in decreasing order of pathogenicity				
	15	29	7	28	34
Acid titre	0·014N	0·011N	0·004N	0·001N	0·013N
Chromatogram					
Oxalate	+	+	+	+	–
Citrate	–	–	–	–	+
Calcium chloride test					
Oxalate	+	+	+	+	–
Citric acid analysis (mg./10 ml.)	0·9	.	.	.	26·0

Isolate 34 produced citric acid instead of oxalic acid. None of these isolates produced either lactic acid or gluconic acid. Following these results further samples of the culture media were tested for the presence of oxalate by the calcium chloride method, giving results similar to those obtained from the chromatogram. An analysis for citric acid carried out on culture filtrates of Isolates 15 and 34 showed the presence of this acid in large quantities in the filtrate from Isolate 34 only. Samples of media from all cultures were titrated against decinormal sodium hydroxide, using phenolphthalein as an indicator, for total acidity. In all these estimations the uninoculated control medium was tested for organic acids and gave a negative response.

The results given in Table 3 show a pronounced positive correlation between the pathogenicity of an isolate and its capacity to produce oxalic acid in culture. The discrepancy with Isolate 34 between the results obtained with bromo-cresol green indicator for acid-producing capacity (Table 2), and later analyses, which show considerable quantities of citric acid produced by this fungus (Table 3), are explained by the fact that citric acid is a much weaker acid than oxalic acid and was not present in sufficient quantities to affect the indicator in the agar.

Experiments were then carried out to test the effects of growth products of pathogenic isolates on groundnut seedling hypocotyls, and also the effects of oxalic acid at strengths similar to those produced by the growth of these fungi. Agar containing growth products was obtained by culturing the fungi on agar in glass tubes (8 mm. diam.), closed at the bottom with a rubber bung, and plugged at the top with cotton-wool. The fungus was cultured in the tubes at one end of the agar, and after 2–3 days' growth at 25° C., the agar was pushed out of the tube and cut into sections 0·5 cm. long, starting from the end with the rubber bung (which was sterile). A thin slice was taken from that end of each section nearer the fungal growth, and examined for the presence of hyphae. The section was also checked for acidity, a pH of about 3 being required. If the section proved to be free of hyphae and was of the necessary acidity, it was used experimentally. Some difficulty was experienced in preparing agar containing the necessary strength of oxalic acid, and a firm gel was not obtained. The resultant loose jelly was used in the same way as sections of agar for other treatments. Two experiments were carried out on seedlings grown in damp sand, using material described above, as well as normal inoculation procedures (four plants per treatment for each experiment). An account of these treatments is given in Table 4 and illustrated in Pl. 11, fig. 2.

Table 4. *Effects of fungal growth products and oxalic acid on groundnut hypocotyls*

Treatments applied to hypocotyl	First experiment	Second experiment
Growth products from Isolate 15	Lesions	Lesions
Growth products from Isolate 29	Lesions	.*
Full inoculation, Isolate 15	Full symptoms	.
Full inoculation, Isolate 29	Full symptoms	Full symptoms
Inoculation with Isolate 29 plus calcium carbonate on hypocotyl and in agar	.	One slight lesion and surface blemishes (ex 4 plants)
0·1 % oxalic acid (=0·008N) in agar	Lesions	.
Sterile water agar	.	Slight blemishes (2 ex 4 plants)
Full inoculation, Isolate 34	.	One slight blemish (ex 4 plants)

* Treatment omitted.

The effects obtained with oxalic acid have been confirmed in further experiments; citric acid in concentrations up to 1·0 % gave very little hypocotyl damage.

These results make it clear that pathogenicity of *A. niger* in Crown Rot is closely connected with the type and quantity of growth products of a given isolate, and that an important component of these products is oxalic acid. Indeed, it seems quite possible that oxalic acid excretion is a necessary condition for the invasion of the groundnut-seedling hypocotyl by *A. niger*. Although previous theories have postulated such a toxic action of oxalic acid as an adjuvant to parasitism (de Bary, 1886; Smith, 1902; Higgins, 1927; Johann, Holbert & Dickson, 1931) yet these theories are open to serious criticism, as Brown (1936) has pointed out. However, the earlier theories were of a more general and speculative nature, and were rejected by Brown for lack of evidence. The more convincing evidence produced here for the role of oxalic acid in the etiology of Crown Rot suggests a reconsideration of the oxalic acid theory for this disease. The theory can explain both the failure to reproduce the disease by application of spores to the hypocotyl, and the success obtained with small pieces of agar culture as inoculum. In particular, the close correlation between oxalic-acid producing capacity and pathogenicity supports the view that oxalic acid plays an important part in primary invasion. The cuticle of the hypocotyl is thin, and, as already shown, does not prevent lesioning either by growth products of *A. niger* in the absence of the fungus, or by 0·1 % oxalic acid. The frequency of *A. niger* in Urambo soil is very high (about 40 % of colonies observed), as results from plating such soil using Warcup's method (Warcup, 1950) show. This results in a high rate of colonization of the emerging cotyledons by this fungus, which may lead to hypocotyl infections by contact, and also in the extensive colonization of organic debris in the soil, which may act as a source of infection.

EFFECTS OF ENVIRONMENTAL FACTORS

For these experiments seedlings were grown in moist sand contained in glass tumblers. The tumblers were kept in constant temperature rooms of the Low Temperature Research Station (Department of Scientific and Industrial Research), under artificial light, and the sand maintained at the desired moisture content by daily watering. Inoculations were made with agar culture disks of *A. niger*, cut with a cork-borer (8·5 mm. diam.) from culture plates that had been grown from a suspension of conidia in the agar medium. This inoculum was applied to the seedling hypocotyl below the level of the sand.

Temperature

The effects of temperature upon growth rate of *A. niger* on agar and upon the development of Crown Rot in inoculated groundnut seedlings were determined as follows. Isolate 15 was grown on a number of different nutrient agars over the temperature range 15–37° C., but as the behaviour was very similar for all agars tested, the data for 2 % dextrose-Dox agar only are presented in Table 5. The acid titre of staled medium of liquid 2 % dextrose-Dox cultures after 138 hr. growth of the fungus at different temperatures is also given.

Table 5. *Effect of temperature upon growth of* Aspergillus niger *on 2 % dextrose-Dox media*

Temperature (° C.) ...	15	20	25	30	37
Growth of *A. niger* on agar (mm./hr.)	0·07	0·13	0·24	0·37	0·43
Acid titre of staled liquid medium after 138 hr. growth	0·007N	0·012N	0·019N	0·026N	0·030N

In order to elucidate the effect of temperature upon disease development, seedlings were treated as follows.

Experiment 1. Raised at temperatures of 20 and 37° C. until ready for inoculation, which was carried out, not simultaneously, but at a certain stage of plant development for each of the two temperatures; after inoculation (and for 1 day beforehand) plants were kept at a uniform temperature of 30° C.

Experiment 2. Raised at a uniform temperature of 30° C.: after inoculation (and for 1 day beforehand) kept at uniform temperatures of 20, 25, 30 and 37° C.

Experiment 3. Raised and maintained at constant temperatures of 15, 20, 25, 30 and 37° C.

In this way, an attempt was made to distinguish between the effect of temperature on predisposition to disease (as shown by effects of pre-inoculation temperatures) and immediate effect of temperature on development of infection (as shown by the effect of variation in post-inoculation temperature). This experimental method for analysis of the effects of environmental factors upon disease was introduced by Foster & Walker (1947). In Table 6 disease ratings were assessed as described in the preceding section.

Table 6. *Effect of temperature on the development of Crown Rot (expressed as disease rating)*

	Post-inoculation temperatures (° C.)				
	15	20	25	30	37
Exp. 1. Pre-inoculation temperatures					
37° C.	.	.	.	89	.
20° C.	.	.	.	46	.
Exp. 2. Pre-inoculation temperature 30° C.	.	85	85	93	92
Exp. 3. Pre-inoculation temperature same as post-inoculation temperature	16	68	67	89	86

The results of Exp. 1 show that plants grown at 37° C. before inoculation were more susceptible to Crown Rot than those grown at 20° C., i.e. that the higher temperature 'predisposed' plants to the disease. The immediate effect of temperature on infection is shown by results of Exp. 2, in which plants had been raised at a uniform temperature of 30° C., before inoculation, and were hence predisposed to the disease, so that the final disease rating was fairly high at all temperatures, though somewhat lower at 20 and 25° C. than at 30 and 37° C. The rate of onset of severe disease symptoms increased markedly with rise of temperature, as might be expected; the rate of mortality at the different temperatures in this experiment is given in Table 7.

In Exp. 3 the seedlings were grown at the same temperatures before and after inoculation. Care was taken to inoculate plants grown at the five different temperatures at the same stage of development in each case, and disease ratings were assessed at suitably graded intervals after inoculation. The observed increase in severity of the disease with rising temperature may be attributed: (1) to the effect of higher temperatures before inoculation in predisposing plants to infection; and (2) to the accelerating effect of higher temperatures on the rate of disease development.

Table 7. *Progressive records of deaths due to Crown Rot (out of 48 plants) in Experiment 2*

Post-inoculation temperature (° C.)	Days after inoculation							
	4	5	7	8	9	12	13	14
37	0	41	42	43	43	·	·	·
30	0	33	40*	·	·	·	·	·
25	0	4	11	26	33	·	36	36
20	0	1	3	8	25	35	38	38

* Plants lost at this date.

Host etiolation

In view of the fact that seedlings used for the experiments on temperature were growing under artificial light in constant temperature rooms, and were hence somewhat etiolated, it seemed desirable to investigate the effect of this factor upon infection. An experiment was therefore set up in which plants grown under normal daylight in a heated glasshouse (mean temperature *c.* 24° C., 17 March to 22 April 1952) were compared with plants receiving a 24 hr. day of illumination from a 40 W. light bulb for the first 8 days after emergence, after which the plants were kept in complete darkness. The plants for this experiment were grown in boxes of a 3:1 sand/loam soil mixture; those receiving weak artificial illumination were severely etiolated by comparison with those grown in normal daylight. Inoculation of the two sets of plants was carried out by the standard method; final disease ratings for normal and etiolated groups were 93 and 100 (means for 22 and 21 plants, respectively), indicating that under the conditions of this experiment a nearly maximum degree of susceptibility was shown by plants grown under full daylight.

Soil texture

Following on the observation that seedlings grown in Kettering loam appeared less susceptible to Crown Rot than those grown in sand, an experiment was carried out to test the effect of soil texture. Seedlings were grown in sand, in 2:1 sand/soil mixture, and in Kettering loam soil, constituting a graded series of soil textures. In order to assess the complicating effect of the more abundant microflora in the undiluted loam soil than in the sand/soil mixture, or in the pure sand, half of the 'soils' in each series were autoclaved for 1 hr. at a pressure of 1 atmosphere before the seeds were planted. The moisture content of each 'soil' was adjusted to 60% of saturation and maintained by daily watering; after planting, tumblers were kept at 30° C. After seedling emergence, plants were inoculated with a pathogenic strain of *A. niger*; final disease ratings (means of 20 seedlings for each treatment) are given in Table 8.

These results show a maximum incidence of Crown Rot in seedlings grown in the sand/soil mixture and in pure sand; in these two media sterilization did not appreciably increase the disease rating, and even in the undiluted loam soil the increase in disease rating due to soil sterilization was only small. It can therefore be concluded that infection by *A. niger* under these conditions is not markedly decreased by the presence of other soil micro-organisms, and that the greater development of the disease in sand and sand/soil mixture is probably due to the improvement of soil aeration.

Soil moisture

Seeds for this experiment were planted in coarse sand, which was brought to different moisture contents (expressed as percentage saturation) by adding water before planting. Plants were raised at 25° C., but were transferred to a temperature of 30° C. at the time of inoculation, just after emergence. Moisture content of the sand was maintained by daily watering. At 20 % saturation, emergence of seedlings was retarded and in some cases did not occur at all. At 30 % saturation, emergence was also slightly retarded but there was no marked reduction in viability of seed. Final disease ratings (means of 32 plants for each treatment) are given in Table 9.

Table 8. *Effects of soil texture and sterilization on the development of Crown Rot*

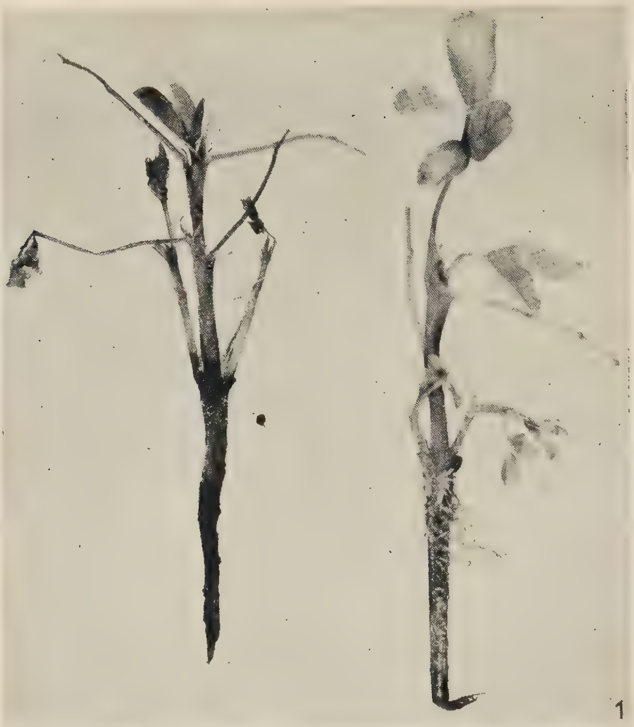
	Disease ratings			
	Sand	2:1 sand/soil	Soil	Mean
Sterilized	98	100	69	89
Unsterilized	95	99	59	84
Mean	97	100	64	.

Table 9. *Effect of soil moisture on development of Crown Rot*

	Percentage saturation of sand			
Crown Rot	20	30	60	80
No. of deaths out of 32 plants	9	31	26	19
Final disease rating (out of 100)	58	99	96	92

These results show that at moisture levels at and above 30 % of saturation, there was little variation in the susceptibility of the host plant to the disease, but as the root environment became wetter, the seedling became less liable to death from such an infection. At moisture levels below 30 %, where gross effects of water lack were observed on the seedling, both disease rating and total mortality markedly decreased.

I wish to thank the Chief Scientific Officer, Dr A. H. Bunting, and the Scientific Staff of the Overseas Food Corporation for their help and encouragement during the initial field work in East Africa, and also Dr S. D. Garrett, under whose supervision the experimental work was carried out at Cambridge. Thanks are also due to the Low Temperature Research Station for assistance with chromatographic studies and for the use of constant temperature chambers; to Dr K. B. Raper for identification of the Aspergilli; to members of the Commonwealth Mycological Institute for identification of other fungi; to Dr A. S. Mann for the citric acid analyses; and to Mr F. T. N. Elborn for the photographs.



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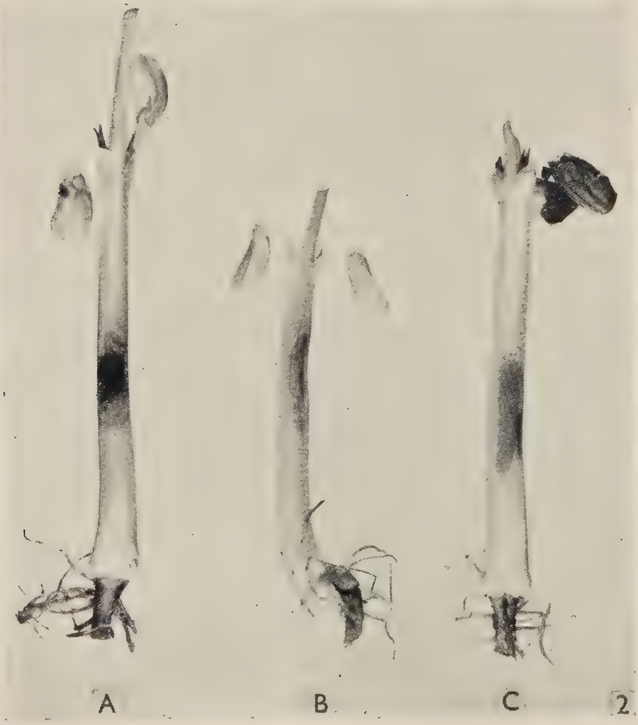

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EXPLANATION OF PLATE 11

Fig. 1. *Left:* groundnut seedling which has died from Crown Rot. *Right:* a groundnut seedling showing signs of recovery from Crown Rot. This reaction is occasionally found in plants suffering from a late and deep infection under conditions where the surface soil remains wet for about 4–5 days. Note growth of small rootlets around the crown. This recovery is rarely permanent.

Fig. 2. A, Crown-Rot lesion obtained by inoculation with agar disk-mycelium inoculum. B, lesion obtained on hypocotyl from agar containing growth products only. C, hypocotyl lesion from treatment with 0·1% oxalic acid agar.

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PHRAGMOCEPHALA IN PURE CULTURE

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(With 4 Text-figures)

The cultural behaviour of *Phragmocephala cookei* and *P. atra* is described. The stilbaceous character of the former is partially lost in culture; that of the latter completely.

In 1951 Mason & Hughes proposed the genus *Phragmocephala* for four species including *P. atra* (≡*Arthrobotryum atrum* Berk. & Br.) and *P. cookei* Mason & Hughes the type species. I obtained the not uncommon *P. cookei* in pure culture in 1946 but the cultural behaviour of *P. atra* was unknown until a fresh collection was made available by M. B. Ellis.

Phragmocephala cookei *in pure culture*

A culture was readily prepared from conidia of a collection on dead stems of *Urtica dioica*, Llanelly, Carms, Wales, 6 August 1946 (Herb. I.M.I. 6152).

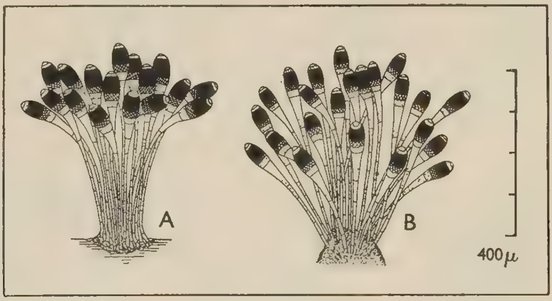

Fig. 1. *Phragmocephala cookei* from Herb. I.M.I. 6152; fructifications: A, from the host; B, from 5-week-old culture on potato-dextrose agar.

Fig. 1 A shows a fructification from the host and Fig. 1 B a fructification from a 5-week-old culture on potato-dextrose agar; successive stages in the development of the conidia are shown in Fig. 2.

After 5 weeks on malt agar a flocculent colony had been produced with the growing edge white and the centre part yellow-brown.

The mycelium is composed of hyaline to very pale brown, smooth-walled, branched, septate hyphae 1–4·5 μ wide. Scattered here and there, or in a circle towards the margin of a colony, aggregations of cells are formed upon which conidiophores develop. Up to 40 conidiophores may arise from a single stroma in the form of a compact or loose tuft with the individual conidiophores quite separate along the whole of their length. The conidiophores are at first more or less cylindrical and pale brown below, increasing gradually in width towards the hyaline apex which becomes blown-out into an oval conidium initial and this is cut off from the conidiophore by a transverse septum. An acropetal succession of five transverse septa is laid down in the initial, and the outer walls of the six unequal cells become variously coloured, subhyaline to almost black.

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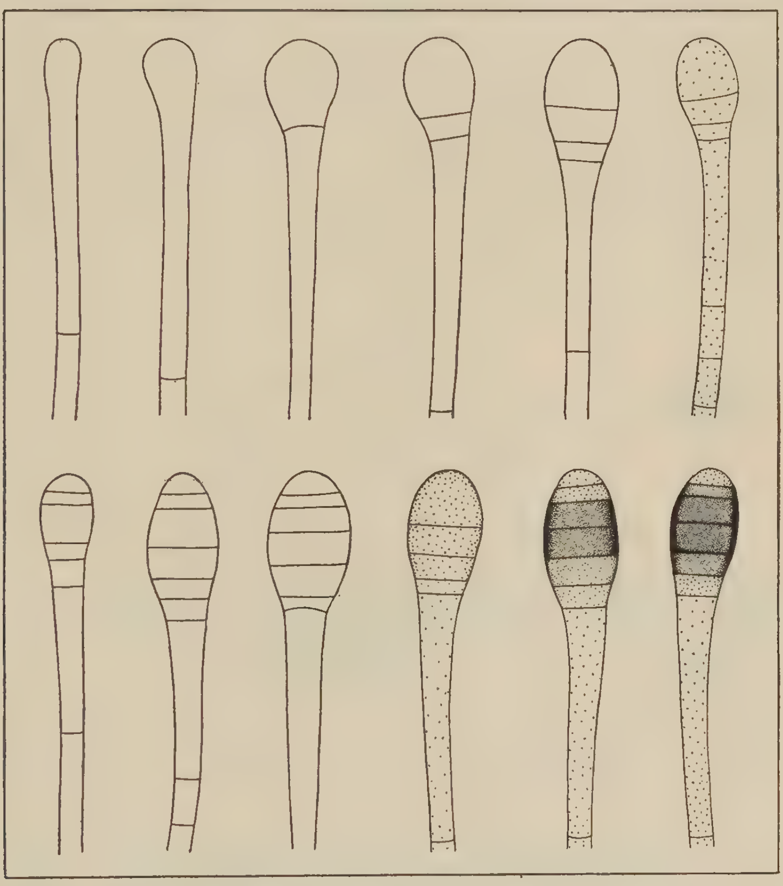

Fig. 2. *Phragmocephala cookei* from Herb. I.M.I. 6152; development of conidia from 5-week-old culture on potato-dextrose agar. × 500.

The two central cells may become almost black but usually they are very dark brown at maturity whilst the cell below them becomes brown or dark brown (occasionally concolorous with the two central ones); the basal cell becomes subhyaline to pale brown, the penultimate cell pale brown and the apical one subhyaline. The conidiophores at maturity are brown and thick-walled below but subhyaline and thinner-walled towards the apex; they are up to 500 μ long, up to 6 μ wide below and 12 μ wide at the apex. The apical cell of the conidiophore is 38–75 μ long.

At maturity the conidia are little different from those produced in nature and which have been described previously.

PHRAGMOCEPHALA ATRA *in pure culture*

A culture was readily prepared from conidia of a collection on dead stems of *Urtica dioica*, Forest of Dean, Hereford, 12 September 1951. (Herb. I.M.I. 47135.)

Fig. 3A shows a fructification from the host and Fig. 3B a fructification from a 5-week-old culture on malt agar; successive stages in the development of the conidia are shown in Fig. 4.

Cultures on steam-sterilized weathered stems of *U. dioica* in tap-water agar had produced no conidiophores after 3 months. On filtered maize meal agar hyaline to subhyaline colonies were produced but no conidiophores after 3 months. However, where a part of the agar had been cut away to expose the glass a few solitary conidiophores were produced from knotted and anastomosing subhyaline to pale brown hyphae which had developed closely adpressed to the surface of the glass.

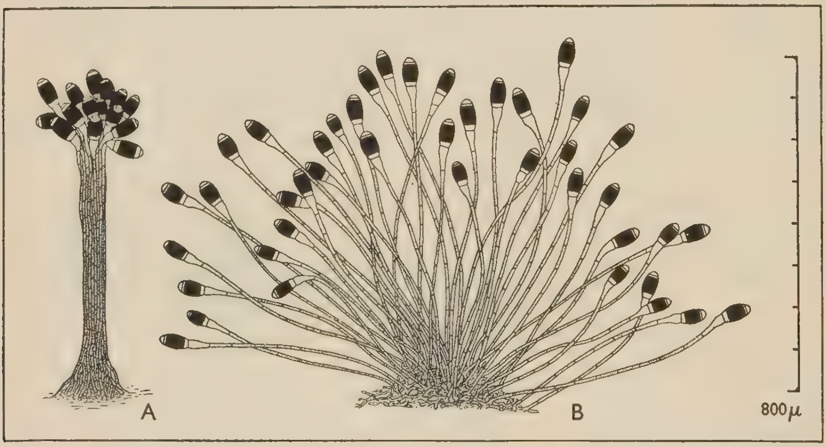

Fig. 3. *Phragmocephala atra* from Herb. I.M.I. 47135; fructifications: A, from the host; B, from 5-week-old culture on malt agar.

On malt agar after 5 weeks a flocculent colony had been produced which, in its deeper parts, was coloured Saccardo's Umber when the reverse was showing through and Cinnamon Buff at its palest; the reverse was Sanford's Brown and Orange Rufous towards the margin. In this same culture groups of conidiophores had matured; they had developed from a tangle of much-branched hyaline to pale brown hyphae.

The fructifications on malt agar are composed of up to 60 conidiophores which are free along the whole of their length. The synnematous character is completely lost, not even an instance of two conidiophores developing in close contact has been seen.

At first the conidiophores are more or less cylindrical and increase more or less abruptly above where the apex becomes blown-out into an oval conidium initial; the initial is cut off from the conidiophore by a transverse septum. An acropetal succession of four transverse septa is laid down and the outer walls of the five unequal cells become variously coloured. The terminal cells in a mature conidium are subhyaline to pale brown, the penultimate one a little darker whilst the two largest cells are very dark brown with a single, central, wide, black equatorial band.

At maturity the conidiophores are flexuous, brown below and subhyaline towards the apex, the apical cell being abruptly swollen towards the distal end; they are up to 1 mm. long, 4–5·5 μ wide for most of their length and up to 12 μ wide at the apex. The apical cell of the conidiophore is 13–35 μ long.

At maturity the conidia are little different from those produced on the host.

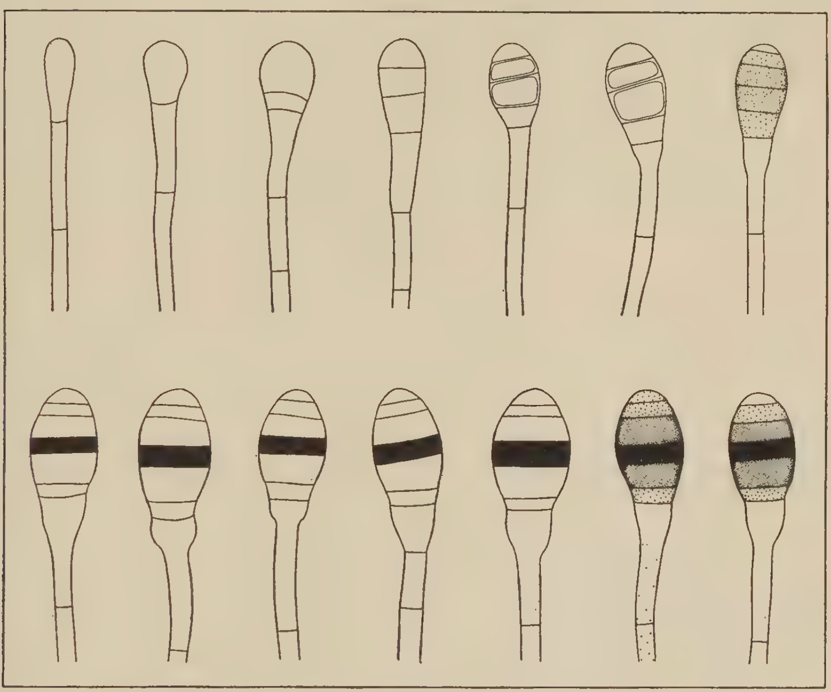

Fig. 4. *Phragmocephala atra* from Herb. I.M.I. 47135; development of conidia from 5-week-old culture on malt agar. × 500.

Scattered sparsely in the aerial mycelium of colonies about 8 weeks old are found pale reddish brown pycnidia. However, most pycnidia are found immersed in the inoculum, consisting of a small lump of agar, in which they are spherical with a many-layered wall, the outer layers being dark brown and thick-walled. By confluence, large, lobed pycnidial cavities may be formed. Lining the pycnidial walls are hyaline flask-shaped phialides which produce hyaline, continuous, oval pycnospores 1·5–2 μ long. I do not know whether these spores are capable of germinating.

Phragmocephala atra was included by Saccardo (1886) with the Phaeostilbeae as *Arthrobotryum atrum* Berk. & Br.; in culture, however, the obvious stilbaceous character of this fungus is lost and a culture would find a place amongst the Dematieae in Saccardo's classification. The breaking down of the closely tufted habit of *Phragmocephala cookei* in pure culture is not so well marked. The two other species included in *Phragmocephala*, *P. minima* Mason & Hughes and *P. setosa* (Berk. & Curt.) Mason & Hughes, produce mononematous conidiophores on their respective hosts and their behaviour in culture is unknown.

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EFFECT OF PARTIAL STERILIZATION AND ACIDIFICATION OF SOIL ON THE FUNGAL POPULATION

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(With Plate 12 and 3 Text-figures)

The effects on the soil fungal population of steam, formalin and acid treatments applied to an old forest nursery soil over Lower Greensand at Ampthill, Bedfordshire, are reported. Samples of soil from the different plots were taken at intervals up to 25 months after treatment, and by plating dilutions, the numbers of fungi per gramme of soil were estimated. Steaming reduced the total numbers of fungi throughout the period tested. The fungi recolonizing steamed plots were varied. Formalin also reduced the numbers but the recolonizing forms were dominated by species of *Trichoderma*. The numbers of fungi increased slightly after acid treatment.

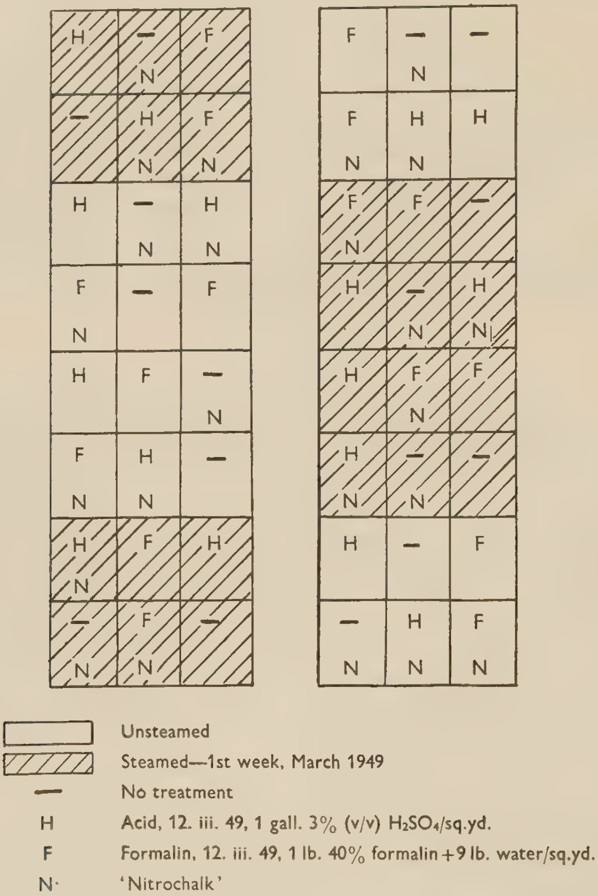
INTRODUCTION

This paper reports results of mycological interest arising from a microbiological and chemical study during 1949–51 of a forest nursery soil subjected to various treatments at Ampthill Forest Nursery, Bedfordshire. The experiments were two of a series carried out over many years on several nurseries by E. M. Crowther, R. G. Warren and B. Benzian of the Chemistry Department, Rothamsted Experimental Station, in association with the Research Branch of the Forestry Commission and under the general supervision of the Forestry Commission's Sub-Committee on Nutrition Problems in Forest Nurseries (Crowther, 1950, 1951).

Earlier work had shown that the growth of Sitka spruce (*Picea sitchensis*) seedlings was often markedly improved, especially in the older nurseries, on soil which had been treated with steam, formalin or acidifying agents. Ampthill Nursery was chosen for the experiments in 1949, because it was near enough to Rothamsted for soil samples to be examined within a few hours of collecting. The nursery had been in use for some twenty years and the more sensitive conifer seedlings usually grew poorly. The soil was derived from drift over Lower Greensand. This paper describes mycological observations on two experiments begun in 1949 and 1950 respectively, on slightly acid soil (pH about 6·0).

EXPERIMENT I

The 1949 experiment was laid out in two long strips, each 64 by 15 ft. (Text-fig. 1) and divided across into two blocks. Each of the four blocks had two main plots. In the first week of March 1949, one plot in each block was steamed for about 20 min. (S), using four grids at a time, each grid having five pipes, 4 ft. long and 9 in. apart buried at about 9 in. A few days later, seed-beds were laid out, each of the main steamed and unsteamed plots having six subplots. Paths were left between the plots but no further measures were taken to separate them. On 12 March two of the subplots in each main plot received a 10% formalin solution at the rate of 1 gall./yd.^{2} (F), two further subplots received a 3% v/v sulphuric-acid solution (H) and the remaining pairs were untreated (O). Sitka spruce seed was sown on 2 April. During the summer one of each pair of subplots with similar pre-treatments received top dressings of 'nitro-chalk' (N).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▨ Steamed—1st week, March 1949
— No treatment
H Acid, 12. iii. 49, 1 gall. 3% (v/v) H₂SO₄/sq.yd.
F Formalin, 12. iii. 49, 1 lb. 40% formalin+9 lb. water/sq.yd.
N· 'Nitrochalk'

Text-fig. 1. Plan of Ampthill Experiment, 1949.

Soil samples were taken on several occasions at a depth of 4 in. from each of the four replicate subplots of the six treatments (O, F, H, S, SF, SH) not receiving nitrogen fertilizer in summer, and these were bulked in pairs to give duplicate laboratory samples A and B for each treatment. Dilutions of 1:25,000 (occasionally also 1:2500) were used for plating on Czapek-Dox medium (pH 4·8) to determine the total numbers of fungi on the untreated plots for comparison with the numbers occurring after the various soil treatments and to gain some idea of the variety of the fungal flora. Five plates were used for each sample of which there were twelve in all, consisting of a duplicated series of samples from six treatments. The plates were counted after incubation at 25° C. for 9 days.

The same series of dilutions were used for studying the bacterial populations of the different plots in work which will be published later by Miss L. M. Crump of the Department of Soil Microbiology.

Sampling began on 20 April 1949, some 6 weeks after steaming, and was repeated in May, June, August and November of that year, and March and August 1950, the final sample being taken in May 1951, 25 months after the beginning of the experiment.

Table 1

PHYCOMYCETES

Mortierella hygrophila Linneman
M. hygrophila var. minuta Linneman
Mortierella spp.
Mucor hiemalis Wehmer
M. ramannianus Möller
Rhizopus [nigricans Ehrenberg] stolonifer (Fr.) Lind.

ASCOMYCETES

Arachniotus candidus (Eidam) Schroeter
Aspergillus (? nidulans (Eidam) Wint.)
Penicillium vermiculatum Dangeard

FUNGI IMPERFECTI

Acrostalagmus cinnabarinus Corda
Aspergillus (? ustus (Bainier) Thom & Church)
Aspergillus spp.
Cephalosporium A series—white mycelium
Cephalosporium B series—pink mycelium
Cephalosporium C series—olive brown mycelium
Cladosporium herbarum Link ex Fr.
Coniothyrium sp. A.
Cylindrocarpon radicicola Wollenw.
Fusarium spp.
Gliocladium roseum (Link) Thom
G. atrum Gilman & Abbott
Gliomastix convoluta (Harz) Mason
Helminthosporium sp.
Monilia geophila Oudemans
Oidiodendron griseum Robak
Paecilomyces sp. A
Paecilomyces sp. B
Penicillium adametzi Zaleski
P. (? frequentans Westling)
P. janthinellum Biourge
P. lilacinum Thom
P. nigricans (Bainier) Thom
P. restrictum Gilman & Abbott
P. spinulosum Thom strain A
P. spinulosum Thom strain B
Penicillium spp.
Phoma eupyrena Sacc.
Pyrenochaeta sp. A
Sporotrichum carnis Brooks & Hansford
Stachybotrys atra Corda
Trichocladium asperum (Corda) Harz
Trichoderma viride Pers. ex Fr.
Trichothecium roseum Link
Verticillium nubilum Pethyb.

Throughout the experiment certain species of fungi proved troublesome by over-growing the plates in 3–4 days, making a final count after the 9-day incubation period impossible and preventing the isolation and identification of many small and slow-growing colonies. Some fungi were identified on the plates directly, but the majority were isolated for later identification. The handling of the large number of different types of fungi obtained at successive samplings soon became overwhelming, and it became impossible to correlate and name so many isolates in the time available. Later, when frequently occurring fungi became more familiar, many rarer forms were of necessity ignored, particularly in the control and acid-treated plots. This account is, therefore, far from complete but is of interest in showing the profound effect that soil treatments have on the fungal population.

Over 120 of the fungi isolated were recognized as distinct species. Many of these could not be named. A list of isolates which were identified is given in Table 1.

The author is indebted to several mycologists for help in naming some of the isolates, and would like particularly to thank Major H. A. Dade, Mr E. W. Mason and their colleagues on the staff of the Commonwealth Mycological Institute for examining cultures of *Arachniotus candidus*, *Cladosporium herbarum*, *Gliomastix convoluta*, *Mucor ramannianus*, *Oidiodendron griseum*, *Phoma eupyrena*, *Sporotrichum carnis*, *Stachybotrys atra*, *Verticillium nubilum*, *Coniothyrium* sp. A., *Paecilomyces* sp. A. and *Pyrenochaeta* sp. A.

Thanks are also due to Mrs Turner of the Department of Botany, the University of Nottingham, for naming *Mortierella hygrophila* and *M. hygrophila* var. *minuta*. Mr G. Smith of the London School of Hygiene and Tropical Medicine kindly identified *Penicillium spinulosum*, *P. restrictum*, *P. janthinellum*, and *P. adametzi*.

The total numbers of fungi in thousands per gramme in samples from the different plots are shown in Text-fig. 2 against the sampling dates, the means of duplicate samples being plotted up to 24 November, after which the duplicates were bulked to give a single laboratory sample.

Control plots (O)

Throughout the experiment the control plots showed a varied fungal flora, giving estimated numbers of 200–308 thousand fungi per gramme of soil. The replicates gave good agreement. The following species and genera were isolated and identified from the control plots:

Cladosporium herbarum	f.*	P. nigricans	o.
Coniothyrium sp. A.	f.	Pyrenochaeta sp. A	o.
Gliocladium roseum	f.	Stachybotrys atra	o.
Paecilomyces sp. A.	f.	Trichoderma viride	o.
Penicillium lilacinum	f.	Verticilium nubilum	o.
P. spinulosum strain A	f.	Aspergillus (nidulans ?)	r.
P. spinulosum strain B	f.	Cylindrocarpon radicicola	r.
P. restrictum	f.	Monilia geophila	r.
Phoma eupyrena	f.	Mucor hiemalis	r.
Acrostalagmus cinnabarinus	o.	Oidiodendron griseum	r.
Gliomastrix convoluta	o.	Penicillium vermiculatum	r.
Mortierella hygrophila	o.	Rhizopus stolonifer	r.
Paecilomyces sp. B.	o.	Tricothecium roseum	r.
Penicillium janthinellum	o.		

One or more species of the following genera were recognized but not identified further:

Cephalosporium spp. A series	f.	Mortierella spp.	o.
Penicillium spp.	f.	Phoma spp.	o.
Cephalosporium spp. B series	o.	Sporotrichum sp.	r.
Fusarium spp.	o.	Verticillium sp.	r.

Throughout the experiment *Penicillium* was the genus most widely represented and *P. spinulosum* the most frequent species on the plates. Whether this reflects its occurrence in the soil or merely its profuse sporulation is not known. It has occurred as two strains. which differed most obviously in colour, the more frequent (strain B) being a more olive-green.

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* The following symbols have been used to give an approximate frequency of occurrence of species: d., dominant; f., frequent; o., occasional; r., rare.

Acid-treated plots (*H*)

In the acid-treated soils the flora was also varied and no particular species was dominant. Acid treatment, which reduced the pH of the soil from 5·8 to 4·6, encouraged the development of fungi and the total numbers are on the whole higher than on the control plots. From these plots the following additional species and genera were isolated:

Cephalosporium (C series)	f.	Helminthosporium sp.	r.
Penicillium spp.	f.	Trichocladium asperum (Corda) Harz	r.
Mucor ramannianus	r.		

A type of *Cephalosporium* appearing at first as a cream-coloured colony but soon developing a submerged brown olive-green mycelium was always represented by several colonies from acid-treated plots but was never isolated from other plots. At least two forms of this *Cephalosporium* type were recognized by a difference in spore size, one having spores about 1·6–4 × 1·4–2 μ and the other 6–8 × 1·4–2 μ.

Pl. 12, figs. 1 and 2, show representative Petri dishes from the control and acid plates, respectively, 10 days after pouring with dilutions from the final sample in May 1951.

Steam-treated plots (*S*)

Steam had almost completely eliminated the fungus population at the time of the first sampling in April. Then, some 6 weeks after steaming, only five colonies appeared on ten plates—*Penicillium spinulosum*, *Coniothyrium* sp. A, an unidentified *Fusarium* and two others. *P. spinulosum* and *Coniothyrium* sp. A were always present in subsequent platings and were joined early by *Cladosporium herbarum*, *Mortierella* spp. and *Phoma eupyrena*, and these remained the most constant isolates in later months. Other identified colonies were: *Gliocladium atrum*, *Paecilomyces* sp. A, *Paecilomyces* sp. B, *Penicillium adametzi*, *P. janthinellum*, *P. nigricans*, *P. restrictum*; all of which appeared several times at the later dates.

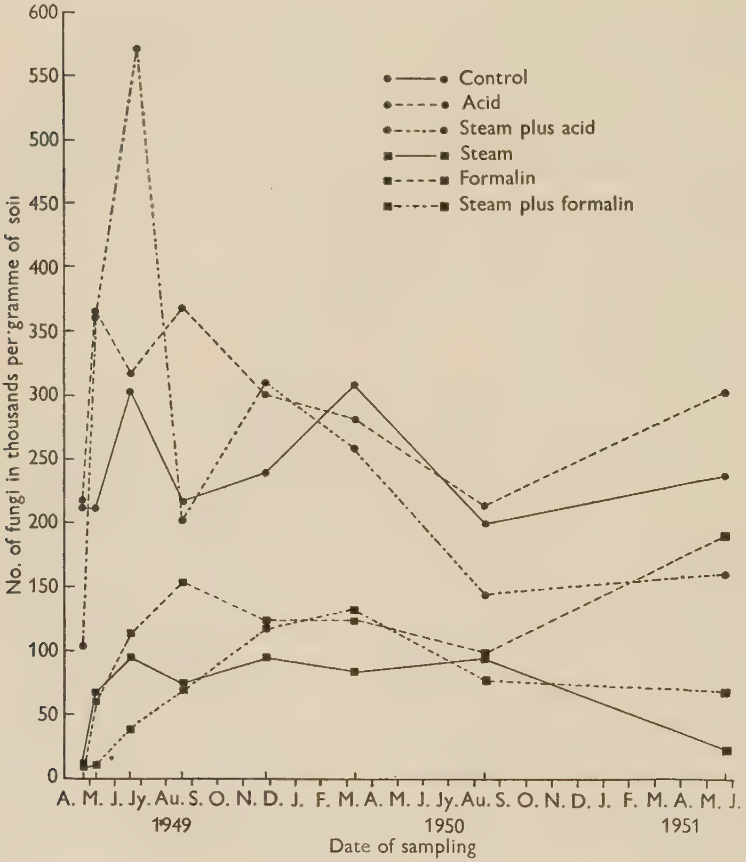
In May 1951, 25 months after the initial sterilization, the total numbers of fungi were still only about half those of the controls (Text-fig. 2). These total count estimates, particularly of the early samples, are based on the occurrence of only a few colonies (sometimes no colonies) per plate. At this final sampling, two of five plates were still blank at a dilution of 1:25,000. Pl. 12, fig. 3, shows a *Fusarium* sp. and *Paecilomyces* sp. A on a third plate, while the remaining two plates carried *Coniothyrium* sp. A, and an unidentified colony respectively. From five plates from a dilution of 1:2500 the following numbers of fungi were identified:

Fusarium sp.	12	P. nigricans	1
Phoma eupyrena	4	P. spinulosum	1
Mortierella spp.	3	Sporotrichum carnis	1
Coniothyrium sp. A	1	Trichoderma viride	1
Mucor ramannianus	1	Verticillium nubilum	1
Penicillium janthinellum	1	Penicillium spp.	Several

The majority, if not all, the recolonizers of the steamed plots are prolific spore-formers and all those identified were present in the control areas.

Formalin-treated plots (*F*)

The most striking feature of this investigation has been the great development of forms of *Trichoderma* on these plots, a dominance which has persisted throughout the period of sampling. *Trichoderma* occurred as various strains, which have not been separated. They are referred to as *T. viride*.



Text-fig. 2.

A *Gliocladium*-like form, at first identified as *G. fimbriatum* Gilman & Abbott, has finally been included under *Trichoderma viride*. It seems similar to forms Warcup (1951 *a*, *b*) isolated from various soils, including an adjacent area at Ampthill, which he called *T. viride*, and Brian (1944, 1951) considered a culture sent to him to be similar to aberrant strains of *T. viride* having a more complex conidiophore than typical forms.

At the first sampling three colonies appeared in ten plates, of which two were *Penicillium spinulosum*. Five weeks later, from a total of thirty-two colonies on ten plates, twenty-eight were *Trichoderma*. Table 2 shows the numbers of colonies of this species compared with the total colony number for all plots except the control. The figures represent the total numbers of colonies found on ten plates (two replicate samples) until March 1950, after which date only one sample was used.

Trichoderma grows quickly, covering a plate in 4 to 5 days, and was a great nuisance. New colonies could not be seen after the 4th day of incubation, and those already observed could not be isolated or identified, as they were overgrown and generally their form was distorted. The total colony numbers in Table 2 should probably be somewhat higher for the F and SF plots and the curves for the plots on Text-fig. 2 derived from them are, therefore, only approximate, for the most part representing the incidence of *Trichoderma* only. Pl. 12, fig. 4, shows a typical plate from the formalin series, showing *Trichoderma* dominating the plates, the darker segment being the *Gliocladium*-like form. How far the F plots have been recolonized by other species is difficult to assess. *Penicillium spinulosum* occurred on one plate in April before *Trichoderma* became established. Other species included: *Aspergillus* (*ustus*?), *Cladosporium herbarum*, *Fusarium* spp., *Gliocladium roseum*, *Paecilomyces* sp. B, *Penicillium lilacinum*, *Phoma eupyrena*, *Penicillium* spp. Plating a dilution of 1:2500 at the final sampling, 102 colonies were counted on five plates on the 4th day of incubation, and of these it was considered that fewer than twenty-five were of fungi other than *Trichoderma*. The mycelium spread and adjacent colonies merged so quickly, that the individual colonies could not be distinguished after that day. The few other colonies identified included *Gliocladium roseum* (2), *Fusarium* sp. (2), *Phoma eupyrena* (1) and several Penicillia.

Steam + formalin plots (*SF*)

Steaming, followed by an application of formalin, initially reduced the fungal population almost to extinction. In April, 5 weeks after treatment, only two colonies of *Penicillium spinulosum* and another *Penicillium* sp. appeared on ten plates. A month later *Trichoderma* appeared, but to a lesser extent than on the plots treated with formalin only (Table 2). It caused the same difficulty in observing other colonies but as late as November, 9 months after treatment, two of ten plates were blank, evidence that the absence of other fungi was not due wholly to their being inhibited or masked by *Trichoderma*, but that re-colonization of the soil was not taking place to any extent. In August 1950 two plates had *Trichoderma* but the remaining three plates had only twelve colonies among them, of which two were Penicillia and two *Coniothyrium* sp. A.

Other species from the SF plots included: *Acrostalagmus cinnabarinus*, *Cladosporium herbarum*, *Gliocladium roseum*, *Fusarium* sp., *Mortierella hygrophila*, *Penicillium* spp., *Pyrenochaeta* sp., *Sporotrichum carnis*.

In May 1951, only twelve colonies appeared on five plates (see Pl. 12, fig. 5). These included:

Coniothyrium sp. A	2	Phoma eupyrena	1
Mortierella sp.	1	Trichoderma (album ?)	1
Penicillium (frequentans ?)	1	T. viride	2

At a dilution of 1:2500 *Trichoderma* was the dominant species, comprising ten of the sixty-two colonies. Other identified colonies were:

Cephalosporium sp.	1	Penicillium lilacinum	1
Coniothyrium sp. A	2	Phoma eupyrena	4
Fusarium sp.	3	Phoma sp.	3
Fusarium sp.	1	Penicillium spp.	Several
Penicillium janthinellum	5	Pyrenochaeta sp. A	1

The distribution of *Trichoderma* on the F, S and SF plots is interesting. In a total of sixty-five plates examined for each treatment for the whole experiment 189 colonies of this species appeared on the F plates, thirty-five colonies on the SF plates and only three on the S plates. The figures for the 1:2500 dilution plated in April 1951 were 75, 10 and 1 respectively.

Table 2. *Total colonies of* Trichoderma *compared with total colonies of all species*

	Formalin		Steam + formalin		Steam	
	Trichoderma	Total spp.	*Trichoderma*	Total spp.	*Trichoderma*	Total spp.
April 1949	0	3	0	3	0	5
May 1949	28	32	7	7	0	24
June 1949	41	41	12	14	0	35
August 1949	48	57	5	26	3	28
November 1949	33	43	6	44	0	33
March 1950*	15	20	1	21	0	14
August 1950*	12	18	2	14	0	17
May 1951*	12	34	2	12	0	4
Total	189	248	35	141	3	160

	Acid		Steam + acid	
	Trichoderma	Total spp.	*Trichoderma*	Total spp.
April 1949	0	78	0	37
May 1949	0	118	11	131
June 1949	4	115	9	199
August 1949	2	134	10	88
November 1949	8	106	1	109
March 1950*	1	51	7	47
August 1950*	0	38	3	26
May 1951*	3	50	0	28
Total	18	690	41	632

* On five plates only.

Steam + acid-treated plots (*SH*)

The acidification of soil already steam-sterilized allowed more rapid recolonization than did steaming alone. The application of acid had decreased the pH of the soil from 5·8–6·0 to 4·6. The first samples showed the total numbers to be considerably above the very low figures for the S plots. This was, in part, due to *Penicillium spinulosum*, which accounted for twenty-one out of thirty-seven colonies on the ten plates. Other colonies recognized were *Aspergillus* (*nidulans*?), *Cephalosporium* B series, *Penicillium adametzi*, *Penicillium* spp.

In June there was a spectacular rise on both replicates to over 500,000 per g. due almost entirely to *P. spinulosum*. Although this species was present on the subsequent sampling dates, it became gradually less dominant and the total numbers of fungi dropped to just below the figures for the controls. Table 3 shows the total number of *P. spinulosum* colonies counted on ten plates and the total number of colonies of all species.

Table 3. *Total colonies of* Penicillium spinulosum *on steam + acid plots compared with total colonies of all species*

	P. spinulosum	Other spp.
April 1949	21	37
May 1949	43	131
June 1949	104	207
August 1949	35	88
November 1949	14	109
March 1950*	9	47
August 1950*	4	26
April 1951*	8	28

* On five plates only.

Trichoderma was represented by a small number of colonies in every sampling after the first. *Cephalosporium* C series, mentioned as only occurring on the acidified plots, was always represented by several colonies. The following fungi were identified on the SH plates.

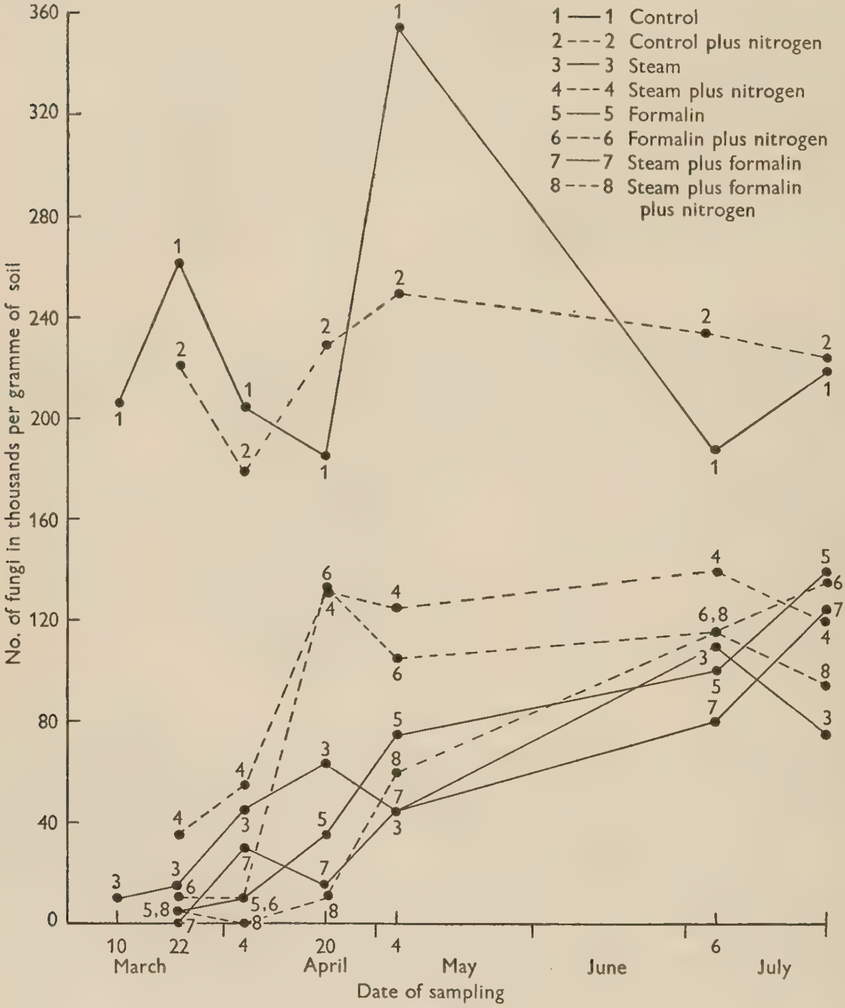
Penicillium spinulosum	d.	Cladosporium herbarum	r.
Cephalosporium, C series	f.	Coniothyrium sp. A	r.
Penicillium spp.	f.	Gliomastix convoluta	r.
Cephalosporium, B series	o.	Mortierella sp.	r.
Penicillium adametzi	o.	Mucor ramannianus	r.
P. restrictum	o.	Oidiodendron griseum	r.
Trichoderma viride	o.	Phoma eupyrena	r.
Pyrenochaeta sp.	o.	Penicillium lilacinum	r.
Arachniotus candidus	r.	P. nigricans	r.
Aspergillus (nidulans ?)	r.	Sporotrichum carnis	r.

Experiment II

In March 1950, a second experiment was set up at Ampthill on similar lines but with soil acidification omitted. Steaming, applications of formalin, both by itself and after steaming, and the untreated control were the four treatments used. To half the number of plots for each treatment 'Nitro-Chalk' was added, giving eight series of plots in all. This time, samples were taken from plots both with and without added nitrogen. Sampling began 3 days after steaming on 10 March 1950 and continued at fortnightly and then monthly intervals until 31 July 1950, seven samples in all being taken. The results paralleled very closely those of the previous year's experiment. The total numbers of fungi per gramme are shown in Text-fig. 3. The addition of 'Nitro-Chalk' seemed to have had no effect on the numbers in the control plots, while the curves for the three treated soils were slightly higher for those plots which had received nitrogen.

The fungi, which were identified on the plates, were, on the whole, the same as those found in the earlier experiment. As before, the earliest recolonizers of the S, F and SF plots, could be found among: *Aspergillus* spp., *Cladosporium herbarum*, *Coniothyrium* sp. A., *Fusarium* spp., *Gliocladium roseum*, *Gliomastix convoluta*, *Mortierella* spp., *Penicillium spinulosum*, *P. nigricans* *Penicillium* spp., *Phoma eupyrena*, *Phoma* sp., *Trichoderma viride.*

The most interesting confirmation of the previous year's experiment was the behaviour of *Trichoderma*. On the first three dates when platings were made, covering the 4 weeks after formalin was applied, only one or two colonies developed on ten plates from the formalin soil samples, and these



Text-fig. 3.

were either *Penicillium* or *Aspergillus*. But on 20 April, about 6 weeks after the application of formalin, *T. viride* appeared and on the three subsequent occasions it was present in increasing numbers. This species was found also in appreciable numbers in the SF plots but appeared only occasionally in the S plots. On the first three sampling dates the thirty plates examined for the F, SF and S plots developed no *Trichoderma* colonies but the subsequent forty plates gave 48, 40 and 13 colonies respectively.

Penicillium spinulosum, though present, was not so frequent, and never became dominant as it had done on the SH plots of the previous year.

This second series of samplings was not continued long enough to confirm the persistent effect of steam and formalin on reducing fungal numbers.

TOLERANCE TO FORMALIN

The early re-invasion and dominance of *Trichoderma* on the F plots but not the S plots suggested that further study of the reaction of this species to formalin might be interesting. In a preliminary experiment 14 g. of air-dry soil was placed in each of sixty-six Petri dishes and autoclaved for 2 hr. These were divided into three series of twenty-two, to which were added 10 ml. of distilled water containing 0·1, 0·05 and 0·01 % formalin, respectively. Five days later two Petri dishes of each series were inoculated in the centre with one of the following: *Cephalosporium* A series, *Cladosporium herbarum*, *Coniothyrium* sp. A, *Mortierella hygrophila*, *Mucor hiemalis*, *Paecilomyces* sp. A, *Penicillium spinulosum*, *Phoma eupyrena*, *Pyrenochaeta* sp. A, *Trichoderma viride* (2 strains); and incubated at 25° C.

Two days later *Trichoderma* had formed definite colonies at all concentrations, though rather smaller at the highest concentration and had spread to the edges of all the Petri dishes by the 6th day. *Coniothyrium* and *Pyrenochaeta* also grew steadily outwards, though less rapidly, on all plates, although the latter was slow to begin growth at 0·1 %. On the 10th day in the 0·1 % formalin plates no growth of *Cephalosporium*, *Mortierella*, *Mucor*, *Paecilomyces* or *Phoma* was observed, and just a trace of *Cladosporium* and *Penicillium*. *Cladosporium* and *Mortierella* had failed also to grow at 0·05 % and there was just a trace of *Paecilomyces*. These results showed that *Trichoderma* was more tolerant of formalin than some of the other frequent isolates found in the present investigation.

Preliminary experiments to measure the effect of different concentrations of formalin on the growth of *T. viride* in sterilized soil or on media were made. These gave variable results, but in one experiment when plain agar was used as the medium and formalin solution added to give concentrations of 0·01, 0·02, 0·04, 0·06 and 0·1 %, growth of one strain estimated as diameter of colony, was appreciably more at the end of 18 days at 0·02 and 0·04 %, than with no formalin and at 0·01, 0·06 and 0·1 %. Germination of the spores, used as inoculum, took place most quickly on the control plates and occurred on successively later days as the concentration of formalin increased. The same tendency was shown once by *Trichoderma* on sterilized soil. However, this would require more critical experiment before any definite conclusions could be drawn, There was some evidence that different strains of *Trichoderma* might react differently and the *Gliocladium*-like strain showed no trace of stimulation by low concentrations of formalin but a steady decrease in colony size as the formalin concentration increased.

DISCUSSION

Selectivity of media and differences in sporulating efficiency of fungi impose great limitations on plating, but for work requiring qualitative as well as quantitative results, it is still a convenient technique. Although the results here do not give the whole picture of the soil fungal population, they do demonstrate the profound effect that disturbance of the normal soil condition has on the fungal flora.

Previous work on the effect of soil sterilization treatments on micro-organisms has been in the main with pot cultures and not in field experiments. The most recent review of the literature is by Hall & Clegg (1949). The slow re-colonization of the plots in the present investigation is in direct contrast to most other work, where total numbers of fungi increased greatly at varying periods after steam-sterilizing soil. Thus, Katznelson & Richardson (1943) found that steaming soil reduced numbers until after 3 months, but that they had increased to a figure above the controls by the 7th month. Tam & Clark (1943) autoclaved soil in pots for 1 hr. at 15 lb. and again 3 days later, and 3–6 weeks after treatment found that the numbers of fungi were very much higher than in the controls. Soil that was autoclaved in pots for 8 hr. and immediately re-inoculated by unsterilized soil sprinkled over the surface gave an enormous increase of fungi over the controls, an increase which was maintained up to 80 days, when the final plating was made (Ludwig & Henry, 1943).

Although soil sterilization does produce effects which can inhibit the growth of some micro-organisms (Katznelson, 1940), most published work reports large increases in total numbers of fungi after treatment. Comparison of the efficiency of sterilization in one experiment with that in another is difficult, when the details of procedure are different, but in the present experiment it may be that the sterilization was more completely lethal to the soil fungi and that re-establishment of an appreciable population, developing either from wind-blown contaminants or from sources unaffected by steam, may take much longer in a field plot than in pot experiments, where the volume of soil is very much smaller.

The early re-colonization of the F plots by *Trichoderma* and its persistent dominance throughout the experiment has been the most striking consequence of the soil treatments. The very rapid growth of *Trichoderma* gives it a great advantage in competition with other fungi, and frequently after soil treatments it becomes the dominant species. Ludwig & Henry (1943) reported that after 8–10 days' incubation it became dominant on autoclaved soil which had been immediately re-inoculated by sprinkling unsterilized soil over the surface. After three soil treatments, formalin, chloropicrin and steam, *Trichoderma* became the most abundant species from rhizosphere samples but not from other parts of the soil (Katznelson & Richardson, 1943). Different plants grown in such treated soils seem to exert some effect on the nature of the population which becomes re-established, but different species of fungi or groups of species might become the prevailing forms in replicate pots (Martin, 1950). A further aid to the frequent dominance of *Trichoderma* is its apparent ability to inhibit or control the spread of some other soil fungi. Slagg & Fellows (1947) found that both *Gliocladium fimbriatum* and *Trichoderma lignorum* [*T. viride*] could inhibit the pathogen *Ophiobolus graminis*, and other evidence is available that the presence of *Trichoderma* in a soil controls or reduces 'damping off' by *Rhizoctonia* and *Pythium* spp. (Allen & Haenseler, 1935; Weindling, 1934), and may protect plants from attack by pathogens such as *Helminthosporium* and *Fusarium* (Anwar, 1949). But how far *Trichoderma* may control the establishment or spread of soil fungi in general is not known, and in laboratory experiments Slagg & Fellows (1947) show that different environmental conditions, such as nitrogen source, may alter the inhibiting potential of one fungus towards another.

The failure of *Trichoderma* to re-colonize the S plots to any extent and its intermediate frequency on the SF plots is curious. This behaviour was repeated in the second experiment. If the re-invading inoculum was airborne, then it must have been equally available to all plots. If, on the other hand, re-invasion came from below, the different penetrative effect of steam and formalin might explain differences in the fungal population of the S and F plots, but scarcely explains the distribution on the SF plots. The evidence of this experiment suggests that there is some relation between formalin and *Trichoderma*. Preliminary experiment showed this species to be more tolerant of concentrations of formalin than some other isolates. This would allow it to become established before other fungi on the F plots and also on the SF plots, but the additional steaming of the latter may have reduced the potential sources of re-infection more than in the F plots. Since air-contamination need not be considered, this indicates that the lethal effect of steaming penetrated deeper than formalin and that it left fewer resistant pockets of infection. This would explain the different degree of re-infection of the F, SF and S plots by *Trichoderma*. A further possibility, of which there was some slight indication, which could account for the distribution is that some low concentration of formalin might actually stimulate the growth of this species, but further investigation of this is necessary. Another factor is that a *Fusarium* sp. which was the most frequent isolate on the steam plates of the final sampling, was antibiotic towards *Trichoderma* on Czapek's medium.

Ecological studies on the effect on the soil fungal population of sterilization treatments similar to those reported were carried out at the same time by Warcup (1951*b*). The experimental area studied by him lay adjacent to the one used for the present investigation at Ampthill. It is of great interest to find that the two main features mentioned in the present paper, viz. the persistent effect of steam treatment in reducing numbers of fungi and the dominance of *Trichoderma* on the formalin plots, were also obtained by him. The most frequently occurring species and earliest re-colonizers of the treated plots found in the two experiments described here are also included in the more extensive lists given by Warcup. Thus, on three occasions on three adjacent areas, steam and formalin sterilization of the soil had a broadly similar and reproducible effect on the fungal population.

The author wishes to express grateful thanks to Dr H. G. Thornton, F.R.S., for his helpful advice, to Mr V. Stansfield for taking the photographs and to Miss Mabel Dunkley for preparing the typescript.

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EXPLANATION OF PLATE 12

Petri dishes photographed 10 days after pouring with dilutions from the final soil samples in May 1951. 1, control; 2, acid; 3, steam; 4, formalin; 5, steam+formalin; 6, steam+acid.

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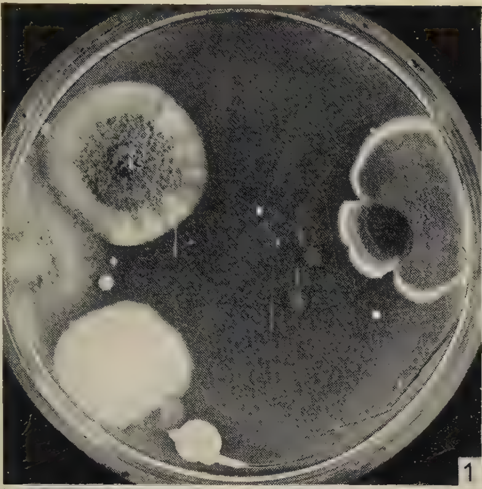
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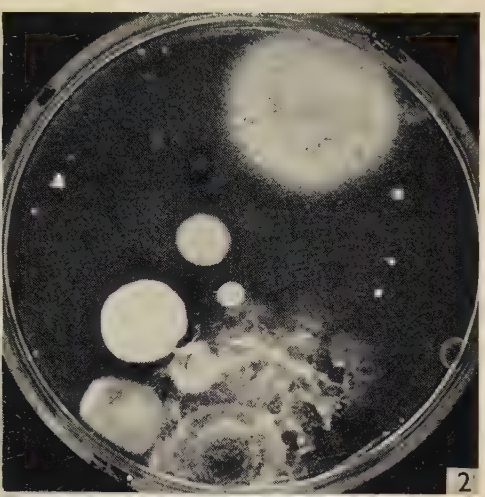
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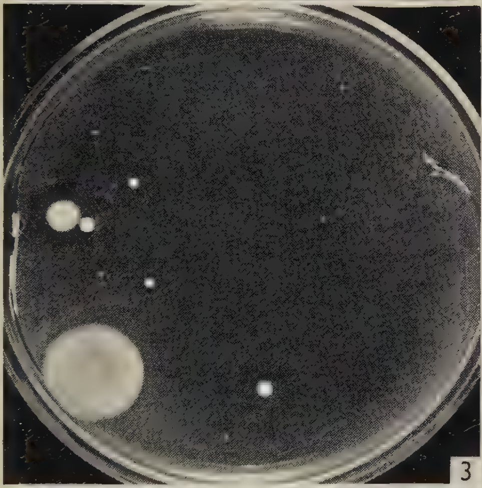
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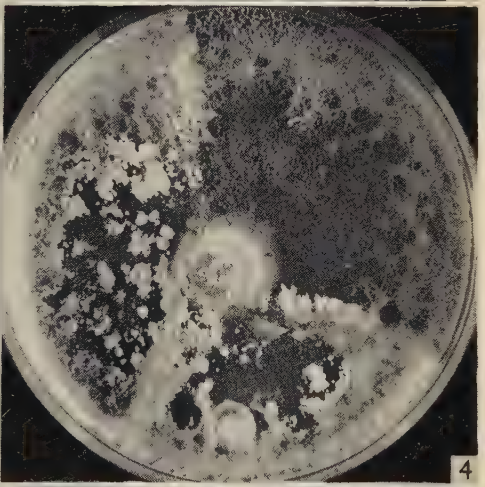
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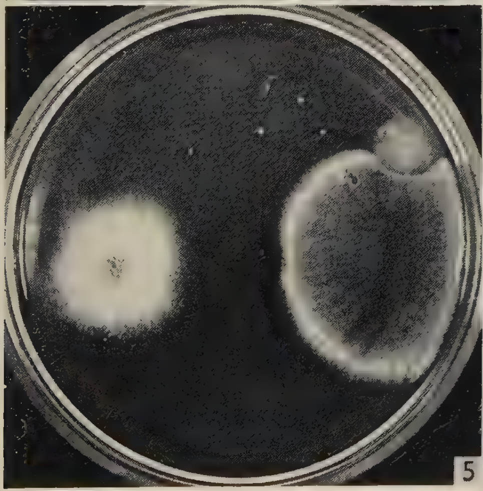
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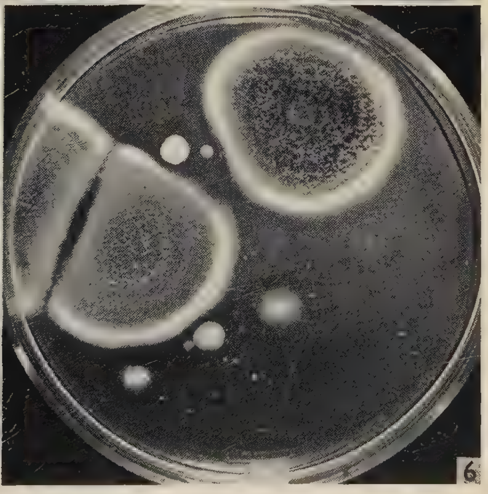
6

TWO NEW SPECIES OF FUNGI ON CLOVE TREES IN THE ZANZIBAR PROTECTORATE

By F. J. NUTMAN and F. M. ROBERTS*

Clove Research Scheme, Zanzibar

(With Plate 13 and 4 Text-figures)

Two species of fungi pathogenic to clove (*Eugenia aromatica*) are described from the Zanzibar Protectorate.

Valsa eugeniae sp.nov. occurs in constant association with clove trees which have died of the Sudden-death disease, and also causes a die-back.

Cryptosporella eugeniae Nutman & Roberts is a severe wound parasite of clove trees of all ages, and often causes the sudden death of young trees.

Two important fungal pathogens have been found associated with clove trees (*Eugenia aromatica*) in the Zanzibar Protectorate. Studies of their pathogenicity are in progress and will be described elsewhere.

Valsa eugeniae Nutman & Roberts, sp.nov.

Conceptacula perithecialia corticem externam occupantia, in primo minuta, orbicularia, truncato-conica, 1 mm. diam., 0·5 mm. alta, nonnihil prominentia, dein saepius secundum rimas peridermii fluendo oblongo-linearia, e matrice confusa plerumque composita, disco omnino carentia, plerumque rostella peritheciorum exserta oculo usurpanda, inferne per zonam cinereo-nigram inter se conjuncta. *Perithecia* in conceptaculo singulo orbiculari 4–12 laxe aggragata, in illis linearibus postremo copiosissima, monosticha, globosa vel e latere compressa, laevia, nigra, 200–280 (240) μ diam., in rostellum cylindraceum 600–900 (780) μ longum (in cortice crassiore etiam fere 5 mm. longum), in medio 50–60 (56) μ latum, apice rotundatum, haud incrassatum producta. *Asci* numerosi, sessiles, laxi, subclavati, utrinque rotundati, tenui-tunicati, apice cingulo refractivo nullo praediti, 8-spori, 17–26 (21) μ longi, 4–6 (4·7) μ lati, in maturitate lumen perithecii omnino occupantes. *Paraphyses* nullae. *Ascosporae* minutae, allantoideae, subdistichae vel inordinate conglobatae, etiam in massa hyalinae vel in extremitate cinereae, 4–5 (4·3) μ longae, 1·0–1·5 (1·3) μ latae.

Pycnidia ut saepius in situ observata, superficialia, sparsa, in disco lignoso caeso trunci vel rami sine ordine disposita, nigra, levia, globosa, interdum masi plana, unilocularia, intus hyalina, postremo invaginationibus labyrinthiformibus, conidiophora gerentibus farcta, 200–300 (250) μ diam. in rostellum 250–500 (340) μ longum, in medio 60–90 (74) μ latum attenuata; cum in ramulis humidis *Ulmi*, tamen, cultivata, pycnidia sub peridermio immersa pluriloculata, et per rostellum emergentia. *Conidiophora* teretia, hyalina, ad instar *Dendrophomatis* ramosa 10–42 (19) μ longa, basi 1·0–1·5 (1·3) μ lata. *Conidia* minuta, allantoidea, acrogena, hyalina, 2–4 (3·4) μ longa, 0·5–1·0 (0·8) μ lata, in globulum melleum denique expulsa. *Hab.* In cortice lignoque *Eugeniae aromaticae* (Myrtaceae) per annum, Zanzibar. *Fungus perithecialis* E. Welsford, 1922 (I.M.I. 14290); A. H. Campbell, Kikangoni Shamba P.T. 30 Nov. 1938 (I.M.I. 44962). *Fungus integer*, J. Nutman et F. M. Roberts, 1951 (I.M.I. 44958 (typus) 44946, 44956, 44957, 44959, 44960, 44961). Obs. Species per perithecia ad instar *Valsae ceratophorae* rostellata, pycnidiaque rostellata, conidiophoris ramosis praedita, et in situ saepius superficialia unilocularia que satis distinguenda.

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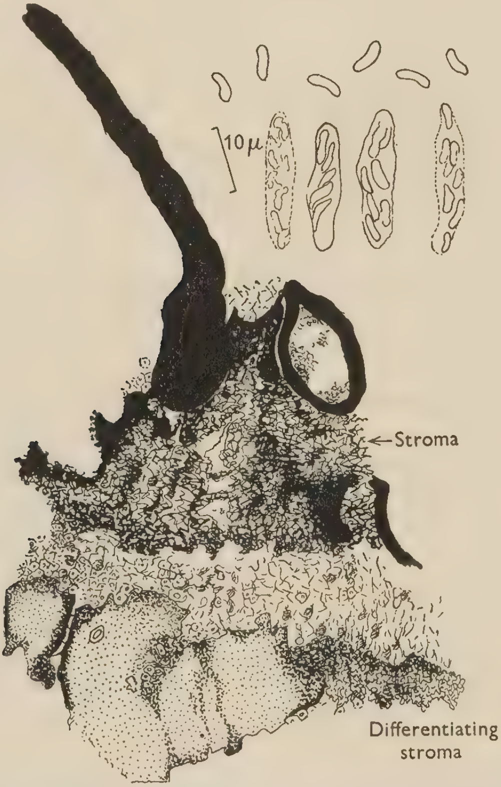
* Seconded from Rothamsted Experimental Station.

The perithecial stromata, ash-grey in colour, develop in the outer bark, initially in the phellogen but subsequently other layers develop (Text-fig. 1). The stromata are fused with the host tissues. The black perithecia sometimes occur in small groups of from 4 to 12, but more frequently in larger groups of 100 or more (Pl. 13, fig. 1). They form in tiers at different levels in the stromata, the oldest externally. As the lower layers of perithecia develop the necks elongate until the tops of all are at approximately the same level. The perithecia are globular or laterally compressed, 200–280 (240) μ in diameter, with long necks 600–900 (780) μ long (up to 5 mm. in thick bark) 50–60 (56) μ in diameter, with a rounded apex.

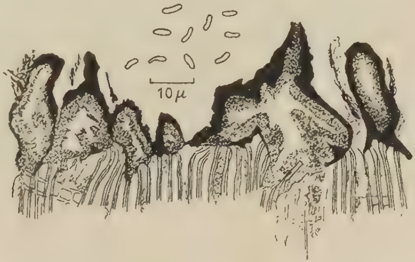


Text-fig. 1. *Valsa eugeniae* n.sp. Perithecia (× 55), asci and ascospores.

The asci are sessile, numerous, loosely aggregated in the perithecium, subclavate, rounded at both ends, the ascus wall not readily visible, 8-spored, 17–26 (21) μ long, 4–6 (4·7) μ wide and eventually fill the lumen of the mature perithecium. Paraphyses are absent. The ascospores are very small, sausage-shaped, hyaline, unicellular, and colourless or opalescent in the mass. They measure 4–5 (4·3) μ long and 1–1·5 (1·3) μ wide.

The pycnidia (Text-fig. 2) are black, flattened at the base, not associated with any definite stromata, unilocular, hyaline internally, with the conidia-producing layer deeply convoluted. They are 200–300 (250) μ in diameter and 28–500 (340) μ high. On elm twig bark, and on clove roots which have been artificially inoculated and cultured in moist chambers the pycnidia may be multilocular (cf. *Cytospora*), but only unilocular pycnidia have been observed in nature.

The conidiophores are hyaline and branched, 10–42 (19) μ long and 1–1·5 μ wide at the base. The pycnospores are small, sausage-shaped, unicellular, 2–4 (3·4) μ long, 0·5–1·0 (0·8) μ wide and are extruded to form slimy yellow globules.



Text-fig. 2. *Valsa eugeniae* n.sp. Pycnidia (L.S. × 60), and pycnidiospores.

Growth in culture

From infected wood in moist chambers, dark brown globules of viscid liquid exude, which when spread on a white surface, reproduce the intense yellow colour characteristic of the diseased wood. Later, pycnidia and perithecia develop freely, the former extruding slimy yellow masses of pycnospores. The colour of the mycelium on the surface of the wood is greenish grey to olivaceous.

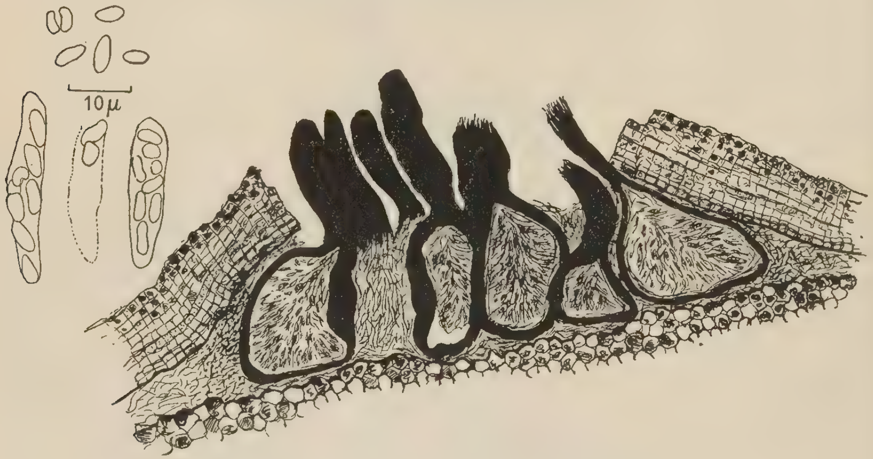
On potato-dextrose agar the mycelium is somewhat scanty, appressed and sometimes sodden, and the colour is yellowish grey to olivaceous. Growth is irregularly zonate, with pycnidia produced in concentric rings. Perithecia have not been produced in culture. On plates a yellow colour often develops, first in the centre but later spreading throughout the medium, in which it is apparently in solution. On the outer edge of the yellow zone there is usually a band of varying width composed of darker greyish mycelium. On agar slopes the surface sometimes becomes deeply convoluted, and pycnidia of relatively enormous size are formed protruding up to 2–3 mm. above the surface. These become crowned with slimy yellow globules of extruded pycnospores.

Field observations

This fungus has been found in the Zanzibar Protectorate in constant association with clove trees which have died of the Sudden-death disease; it also frequently occurs, particularly in some districts, as a rather weak wound parasite of branches. It has not yet been found on any other host. The presence of the fungus is always associated with a brilliant saffron-yellow water-soluble stain in the affected wood which is separated from the healthy by a narrow zone stained bluish grey, and often limited by a sharp black zone-line.

In the field, perithecia first appear at the collars of standing clove trees, not less than 3 months after they have died of Sudden-death, and later can be found in enormous quantity on all the external parts of the trunk and larger branches. They have not been found below ground but will develop if roots are exposed. The black necks of the perithecia at first protrude along cracks in the bark, giving the fructifying area an almost chequered appearance. Later they are found massed in somewhat square or oblong groups (Pl. 13, fig. 1). They also form on the exposed wood of stumps of felled trees, replacing the pycnidia which are the first fruiting bodies to develop in these conditions. The perithecial necks can vary greatly in length from less than 1 mm. to almost 1 cm. Perithecia continue to be produced on standing or felled dead trees for several years, or until the bark disintegrates.

Pycnidia are usually seen on cut, split, or broken surfaces of wood, and they occur in abundance only in moist conditions (Pl. 13, fig. 2).



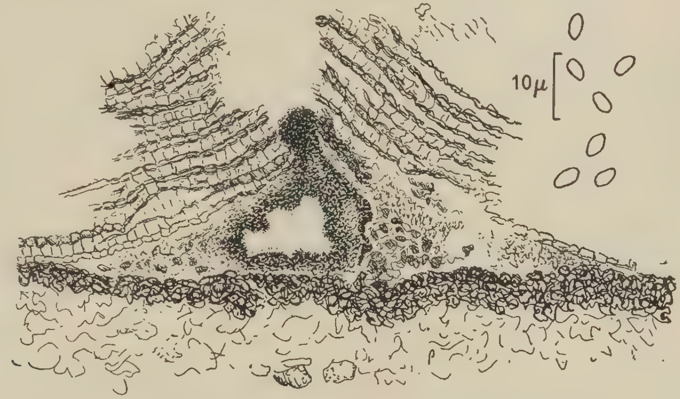
Text-fig. 3. *Cryptosporella eugeniae* Nutman & Roberts. Perithecia (L.S. × 55), asci and ascospores.

Cryptosporella eugeniae Nutman & Roberts (*Ann. appl. Biol.* **39**, p. 307, 1952)

In naturally infected wood the perithecial stromata are valsiform, containing 2–10 perithecia with converging necks (Text-fig. 3). The perithecia often occur in well-defined linear fissures in the bark. They are 500–900 μ in diameter, with necks 470–680 μ long and 120 μ wide when formed under field conditions. When developed in moist chambers, however, the necks can be very much longer. The asci, which are slightly pointed at one end, are sessile, hyaline, 8-spored, and completely fill the lumen of the perithecium; they measure 24–30 (27) μ by 4·5–6 (5·3) μ. The ascus wall is not readily visible. The ascospores measure 7–8 μ by 3–4 μ, are oval to round, unicellular and hyaline. Paraphyses are absent.

Pycnidia also occur in rows, and, on the whole, in smaller individual groups (Pl. 13, fig. 4). The stromata contain unilocular pycnidia $\frac{1}{2}$–$\frac{3}{4}$ mm.

in diameter, with necks up to 1 mm. long. The conidia-producing layers are often deeply convoluted (Text-fig. 4). The conidiophores are unbranched, ampulliform, hyaline, 8–12μ long, and 2–3μ wide. Only one form of pycnospore is produced. This is oval, hyaline, unicellular, 3·4–5μ long and 1·5μ wide and yellowish orange in mass.



Text-fig. 4. *Cryptosporella eugeniae* Nutman & Roberts. Pycnidium (L.S. × 55), and pycnidiospores.

Growth in culture

Pycnidia are formed within 10 days on infected wood in moist chambers. Perithecia may develop much later, and in these conditions sometimes have relatively long necks.

On potato-dextrose agar in pure culture the mycelium is mainly superficial, raised, cottony, and growth is markedly zoned. While the colour is usually a reddish orange, individual cultures vary widely from almost colourless with a faint ochreous tinge to a bright carrot colour or a dark orange red. The colour is more intense on the under side of the culture. Pycnidia are produced freely on plates in concentric rings and sometimes, especially in tubes, may develop to several times the size of those found in nature. In these conditions they extrude orange-yellow masses of spores. Perithecia have not been produced in pure culture, and both ascospore and pycnospore isolations give rise only to pycnidia-producing cultures.

Field observations

This fungus is a widely distributed and severe wound parasite on the clove tree *Eugenia aromatica*, and has twice been found on *E. jambosa*. It is the main cause of dieback of individual branches, and eventually of whole trees, of all ages, and of the so-called 'Sudden-death' of young trees (Nutman & Roberts, 1952).

The fungus ramifies in both wood and bark, where it causes a very distinct and characteristic red-brown discoloration (Pl. 13, fig. 3). Externally its presence can be detected by the fructifications which develop on the bark a short time after infection. At first these are always pycnidia; perithecia are found more rarely and develop later than the pycnidia.

When a large branch or a main trunk is affected often the only external sign is yellow tendrils of pycnospores which exude from cracks in the bark. On smaller branches with thinner bark the dark brown necks of the pycnidia protrude giving a prickly appearance. This is the more conspicuous as the surface of the fructifying area is often coloured a dull orange-brown by the extruded pycnospores (Pl. 13, fig. 4).

We wish to thank Mr E. W. Mason, M.A., of the Commonwealth Mycological Institute, for the great help he has afforded us in this work and for providing the Latin diagnosis, and Dr P. H. Gregory for helpful criticism and advice on preparing the manuscript.

REFERENCE

Nutman, F. J. & Roberts, F. M. (1952). Acute die-back of clove trees in the Zanzibar Protectorate. *Ann. appl. Biol.* **39**, 599–608.

EXPLANATION OF PLATE 13

Fig. 1. Perithecia of *Valsa eugeniae* n.sp. in bark of trunk of mature clove tree which had died some months previously of the Sudden-death disease. Nat. size.

Fig. 2. Cut end of stump of dead clove tree showing pycnidia of *Valsa eugeniae* n.sp.

Fig. 3. Trunk of clove tree affected by die-back slashed to show stain caused by *Cryptosporella eugeniae*.

Fig. 4. Pycnidia of *Cryptosporella eugeniae* in bark of clove tree. × ½ approx.

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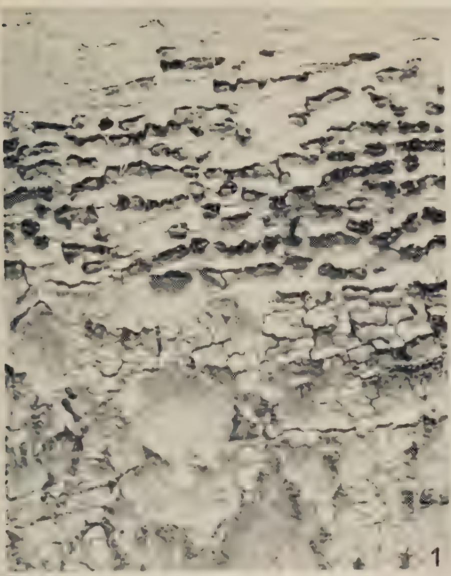
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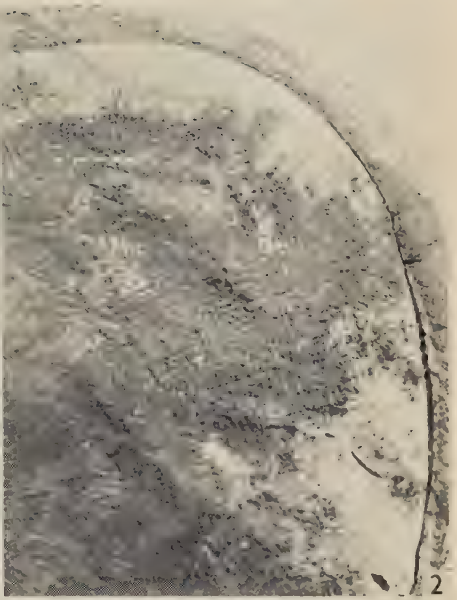
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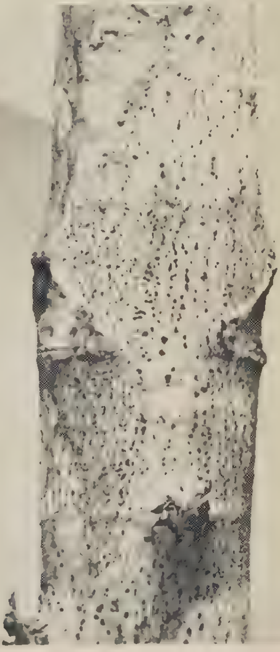
4

PESTALOTIOPSIS FROM THE GOLD COAST AND TOGOLAND

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(With 3 Text-figures)

The following species and variety of *Pestalotiopsis* from the Gold Coast and Togoland are described: *P. dichaeta*, *P. adusta*, *P. neglecta*, *P. disseminata*, *P. guepini*, *P. guepini* var. *major* n.var., *P. hughesii* n.sp., *P. mangiferae*, *P. palmarum*, *P. theae* var. *minor*, *P. maculiformans*, *P. angusta* n.sp., *P. aquatica*. The two new species and the new variety are illustrated.

Mr S. J. Hughes, of the Commonwealth Mycological Institute, Kew, was very fortunate in his collections of *Pestalotiopsis* during April, May and June of 1949; twenty-four collections include thirteen species and varieties, which are relatively high numbers considering the short collection period. It can be concluded that *Pestalotiopsis* spp. are rather frequent plant contaminants, but the lack of host specificity is indicative more of saprophytism than parasitism. They are frequently observed associated with other fungi.

I feel indebted to Mr Hughes for having submitted his collections to me and I am glad to dedicate to him a rather outstanding species to perpetuate his short but very fruitful field work in the Gold Coast and Togoland.

In studying these collections the same procedure has been followed as in my previous studies with this genus (*Bull. Jard. bot. Brux.* **9**, 285–354, 1949; *Trans. Brit. mycol. Soc.* **36**, 81–89, 1953). The localities are indicated precisely in the map on p. 2 in 'Fungi from the Gold Coast and Togoland, I'—*Mycol. Pap.*, no. 48, 1952, by S. J. Hughes.

Section: BISETULATAE

Pestalotiopsis dichaeta (Speg.) Steyaert, in *Bull. Jard. bot. Brux.* **19**, 308, 1949

Pestalotia dichaeta Speg., in *An. Mus. nac. B. Aires*, Ser. III, **13**, 411, 1910.

Macules indefinite. *Acervuli* epiphyllous, scattered, round or elongated. *Conidia* fusoid, more or less constricted at septa, 19–*21·7*–23(25) μ × (5)6–*6·4*–7(8) μ; 3 middle cells (12)13–*14·5*–16(17) μ long, concolorous, sepia or the two upper cells slightly darker; end cells hyaline, the upper conoid bearing 3 or 2 fine spreading setulae, (7)8–*10·9*–13(16) μ long, basal conoid with a pedicel 0–*2·5*–4 μ long.

Presence of abnormal 3-septate conidia with only 2 coloured middle cells. Setulae 1–3, sometimes ramified.

On dead leaves of *Phoenix reclinata* Jacq.: Elmina, Gold Coast (Colony); 7 May 1949 (BR.) (I.M.I. 38502 *f*).

The position of this species in the Bisetulatae is doubtful but it is best kept here until the genus is thoroughly revised.

Section: TRISETULATAE. Subsection: CONCOLORES

Group: Fusiformes. Subgroup: non-spathulatae

Pestalotiopsis adusta (Ell. & Ev.) Steyaert, in *Trans. Brit. mycol. Soc.* **36**, 82, 1953

Pestalotia adusta Ell. et Ev., in *J. Mycol.* **4**, 51, 1888.

Macules large, marginal or involving up to more than half of the leaf, usually at the tip, greyish brown with a well-defined sinuate brown edge. *Acervuli* amphigenous, round, exuding black masses of conidia spreading on the leaf surface. *Conidia* fusiform, 16–*19·1*–21 μ × 6–*6·4*–7 μ; 3 middle cells concolorous, reddish brown, slightly constricted at the septa, 10–*12·6*–14 μ long; end cells hyaline, the upper short conoid bearing 3, rarely 2, spreading setulae 5–*10·5*–16 μ long, basal cell conoid with a pedicel 0–*2*–4 μ long.

On living leaves of *Hippocratea* sp.: Takoradi, Gold Coast (Colony); 10 April 1949 (BR.) (I.M.I. 45095).

Pestalotiopsis neglecta (Thuem.) Steyaert, in *Trans. Brit. mycol. Soc.* **36**, 83, 1953

Pestalotia neglecta Thuem., in *Instituto, Coimbra*, **27**, 326, 1880.

Macules centrifugal, elongated along leaf costa, straw coloured, margined by a thin brown zone. *Acervuli* hypophyllous, round or elongated, scattered. *Conidia* fusoid, 17–*21·4*–24 μ × (5)6–*6·3*–7(8) μ; coloured cells slightly constricted at the septa, with thin walls, finely guttulate, the lower cinnamon, the upper slightly darker, mainly the central one, 11–*13·9*–16 μ long; apical cell long conoid bearing 3 fine, divergent setulae 7–*11·1*–15 μ long, basal cell conoid with a short pedicel (0)2–*3·3*–6 μ long.

On leaves of *Borassus aethiopum*, Mart.; Esiama, Gold Coast (Colony); 14 May 1949 (BR.) (I.M.I. 37943 *b*) (Gold Coast Myc. Herb. 164 *b*).

Pestalotiopsis disseminata (Thuem.) Steyaert, in *Bull. Jard. bot. Brux.* **19**, 319, 1949

Pestalotia disseminata Thuem., in *Instituto, Coimbra*, **28**, 501, 1881.

(*a*) *Macules* indefinite, associated with other fungi. *Acervuli* in irregular scattered colonies. *Conidia* fusiform, straight, 20–*23·8*–28 μ × 5–*6·5*–8 μ; coloured cells light buff, concolorous, slightly constricted at the septa, 12–*13·7*–19 μ long; end cells hyaline, the apical conoid bearing 3, sometimes 2, thin spreading setulae, 9–*15·5*–22 μ long; the basal long conoid with a pedicel 3–*4·7*–8 μ long.

On dead leaves of *Caffea arabica* L.; Aburi, Gold Coast (Colony); 25 April 1949 (BR.) (I.M.I. 38055 *b*) (Gold Coast Myc. Herb. 187 *b*).

(*b*) *Macules* of discoloured areas merging gradually into healthy tissue. *Acervuli* gregarious, more or less in line with leaf nervules, producing minute, dense globular heads of conidia. Conidia as above, (19)22–*24·5*–30(33) μ × 6–*6·8*–8 μ; coloured cells 13–*15·5*–19 μ; 3, sometimes 2, setulae (7)10–*17·6*–30 μ long; pedicel 2–*4·7*–8 μ long. Some coloured cells inconspicuously guttulate.

On dead leaves of *Elaeis guineensis* Jacq.; Apremdo (near Takoradi), Gold Coast (Colony); 11 May 1949 (BR.) (I.M.I. 38616*a*) (Gold Coast Myc. Herb. 290); Asuansi, Gold Coast (Colony); 9 June 1949 (BR.) (I.M.I. 38625*b*) (Gold Coast Myc. Herb. 298*b*).

Pestalotiopsis guepini (Desm.) Steyaert, in *Bull. Jard. bot. Brux.* **19**, 312, 1949

Pestalotia guepini Desm., in *Ann. Sci. nat.*, Ser. II, **13**, 181, 1840.

(*a*) *Macules* large, irregular, grey on upper surface, cigar brown on the under surface. *Acervuli* amphigenous but mostly hypophyllous, round, exuding black masses of conidia around the acervuli. *Conidia* long fusiform, 21–*25·5*–30 μ × 5–*6*–7 μ; 3 middle cells concolorous, brown olivaceous, guttulate, 14–*16·4*–19 μ long; end cells hyaline, the apical subcylindric, bearing 3 thin setulae 18–*24·75*–34 μ long; the basal conoid with a pedicel 4–*6·2*–9 μ long.

On living leaves of *Hippocratea* sp.: Hohoe, Togoland; 28 May 1949 (BR.) (I.M.I. 44045*b*).

(*b*) *Macules* irregular, more or less round, 0·5–1·5 cm. in diameter, cigar brown, scattered. *Acervuli* scattered. *Conidia* as above, 23–*25·8*–29 μ × 6–*6·7*–7 μ; 3 middle cells 15–*16·8*–19 μ long; 3 setulae (15)18–*23·2*–29 μ long, sometimes with a more or less conspicuous lumen; pedicel 3–*5·1*–7 μ long.

On living leaves of *Jatropha curcas* L.; Amanase, Gold Coast (Colony); 28 April 1949 (BR.) (I.M.I. 45130*b*).

Both specimens have narrower conidia with slightly shorter pedicels than in holotype.

Pestalotiopsis guepini (Desm.) Steyaert var. *major* Steyaert var. nov.

Conidia majora quam ea typi, 28–*32*–36(39) μ × 7–*8*–9 μ; setulae 3, interdum 1–*2*–4, rectae, patulae, (13)20–*30*–45(54) μ longe; pedicel (0)3–*7·2*–13 μ longe. (Fig. 1).

On dead leaves of *Encephalartos barteri* Carruth. ex Miq.; Aburi, Gold Coast (Colony); 3 May 1949 (BR., holotype) (I.M.I. 42315*a*, isotype) (Gold Coast Myc. Herb. 600*a*).

Pestalotiopsis hughesii Steyaert sp.nov.

Maculae indefinitae. *Acervuli* circulares vel ovaliformes, 250–500 μ diam. *Conidia* fusoidea, recta vel leniter curva, 4-septata, 34–*38·7*–45 μ × 7–*9·1*–11 μ: cellulae 3 mediae brunneofulvae, concolores, guttatae, 18–*26·8*–32 μ longe, cellulae extima hyalinae, apicalis longe conoides, 2–*3* setulas crassas, lumine definite visibili, 9–*17*–29 μ longe gerens, cellula basalis conoides in pedicello (0)3–*4·2*–8 μ desinens (Fig. 2).

On dead stems of *Cyperus articulatus* L. det. Mr E. Nelmes; Big Ada, Gold Coast (Colony); 30 April 1949 (BR., holotype) (I.M.I. 44237*a*, isotype) (Gold Coast Myc. Herb. 830*a*).

A very characteristic species. The coloured cells each contain a big refringent mass that stains deeply with cotton blue. The setulae are very thick at the base with a thick membrane that thins out suddenly towards the middle of the setula. This feature gives them a more or less bulbous appearance at the base. A wide lumen is visible the whole length. The setulae are sometimes ramified towards the base.

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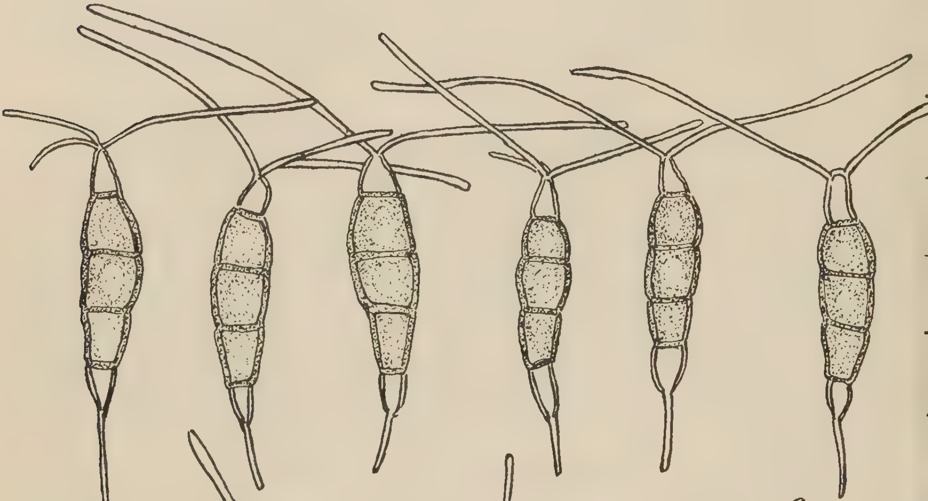

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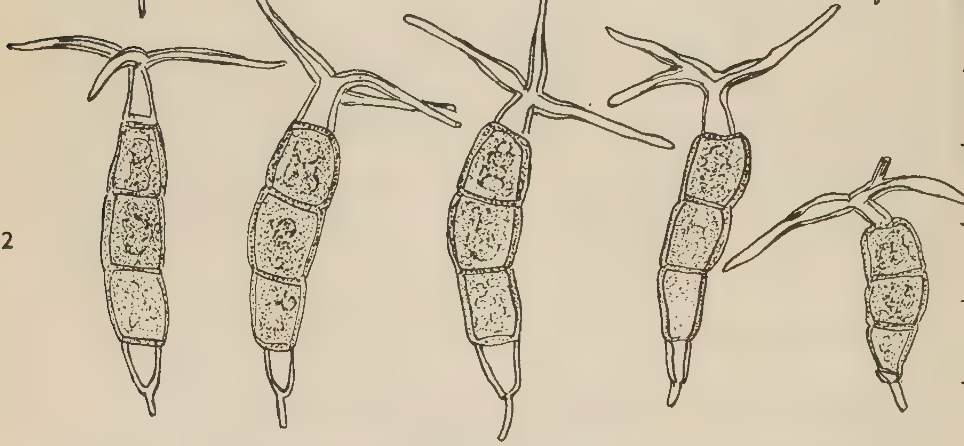

Fig. 1. *P. guepini* (Desm.) Steyaert var. *major* (Steyaert) Steyaert: conidia on *Encephalartos barteri* (I.M.I. 41315*a*); × 1000.

Fig. 2. *P. hughesii* Steyaert: conidia on *Cyperus articulatus* (I.M.I. 44237*a*); × 1000.

Subgroup: Spathulatae

Pestalotiopsis mangiferae (P. Henn.) Steyaert, in *Bull. Jard. bot. Brux.*, **19**, 320, 1949

Pestalotia mangiferae P. Henn. apud De Wild., in *Ann. Mus. Congo belge*, Bot. Ser., v, **2**, 102, 1907

(*a*) *Macules* marginal and centripetal, involving a large leaf area, light brown with a sharply defined, dark brown margin. *Acervuli* amphigenous, regularly spaced but restricted to certain areas of the macules, erumpent, flat, producing a dark greyish mass of conidia. *Conidia* fusoid, straight, 19–*20·3*–22 μ × 5–*5·5*–6 μ; coloured cells brownish cinnamon, slightly constricted at the septa, with rather thick walls, 12–*13*–14 μ long; apical cell conoid bearing 3, sometimes 2, setulae 5–*10·8*–14 μ long; basal cell conoid with a pedicel 2–*3*–5 μ long.

On leaves of *Mangifera indica* L.; Techiman, Gold Coast (Ashanti); 16 April 1949 (BR.) (I.M.I. 37133).

(*b*) *Macules* idefinite, associated with other fungi. *Acervuli* elliptical, circa 300 μ in diameter, regularly dispersed on parts of the macules. *Conidia* as above, 16–*18·8*–21 μ × 5–*5·8*–7 μ; coloured cells 10–*12·2*–14 μ long; setulae 3, sometimes 2, (5)8–*10·7*–18(24) μ long; pedicel 1–*4·7*–5(7) μ long.

On dead leaves of *Elaeis guineensis* Jacq.; Ada, Gold Coast (Colony); 30 April 1949 (BR.) (I.M.I. 38789) (Gold Coast Myc. Herb. 313).

(*c*) *Macules* elongated along mid-rib, grey-brown with deep brown well-defined margin. *Acervuli* amphigenous, small, circular, about 150 μ diameter, spreading its conidia over leaf surface. *Conidia* fusoid, straight, 18–*19·7*–21 μ × 5–*6*–7 μ; coloured cells brownish cinnamon, 11–*12·6*–14 μ long, the middle cells sometimes a little darker; end cells hyaline, the upper conoid bearing 3, rarely 2, spreading setulae, sometimes slightly spathulate, 7–*11·25*–16 μ long, basal cell conoid with a pedicel 2–*3·5*–6 μ long.

On living leaves of *Hyphaene thebaica* Mart.; Achimota, Gold Coast (Colony); 27 June 1949 (BR.) (I.M.I. 45083*e*).

Pestalotiopsis palmarum (Cooke) Steyaert, in *Bull. Jard. bot. Brux.* **19**, 322, 1949

Pestalotia palmarum Cooke, in *Grevillea*, **5**, 101, 1877

(*a*) *Macules* circular or affecting up to half the leaf surface, deep red-brown, greyish at the edge and surrounded by a blackish brown margin on the under leaf surface, grey reddish brown on the upper surface. *Acervuli* mostly epiphyllous, superficial under the epidermis of which a small disk soon falls out liberating the conidia. *Conidia* fusoid, 18–*21·5*–25 μ × 5–*6*–7 μ; coloured cells cinnamon brown, constricted at the septa, 11–*13·75*–17 μ long; end cells hyaline, the upper conoid, bearing 2 or 3 rarely spathulate setulae, basal cell conoid with a pedicel 0–*3·6*–5 μ long.

On living leaves of *Diospyros* sp.; Weija, Gold Coast (Colony); 24 April 1949; (BR.) (I.M.I. 43715*a*).

(*b*) *Macules* irregular, affecting large leaf areas, often marginal, with a sharply defined margin but surrounded by a diffuse yellowish colour. *Acervuli* amphigenous, scattered, or more or less aggregated. *Conidia* fusoid, 18–*22·5*–26 μ × 5–*6·4*–8 μ; coloured cells often guttate, 12–*15·1*–18 μ long; end cells hyaline, conoid, the upper bearing 3, sometimes 2, occasionally ramified, spreading, spathulate setulae, 8–*14*–21 μ long, the basal with a pedicel 0–*3·8*–8 μ long.

On living leaves of *Desplatzia lutea*; Anomabu, Gold Coast (Colony); 15 May 1949 (BR.) (I.M.I. 43938*a*) (Gold Coast Myc. Herb. 795*a*).

(*c*) *Macules* marginal, diffuse at the edge. *Acervuli* small, circular, erumpent. *Conidia* fusoid, 19–*21·6*–24(30) μ × 5–*6·2*–7 μ; coloured cells 13–*15*–16 μ long, the middle cell often the smallest; end cells hyaline, the upper bearing 3, sometimes spathulate, setulae 11–*16*–24 μ long, the basal with a pedicel 2–*3·5*–5 μ long.

On living leaves of unknown host; Abe, Gold Coast (Colony); *S. J. Hughes* 640, 15 May 1949 (BR.) (I.M.I. 44583*c*).

Pestalotiopsis theae (Saw.) Steyaert var. *minor* (Steyaert) Steyaert, in *Bull. Jard. bot. Brux.* **19**, 327, 1949

Pestalotia theae Saw. var. *minor* Steyaert, in *Bull. Jard. bot. Brux.*, **19**, 184, 1949

(*a*) *Macules* indefinite. *Acervuli* round, erumpent, producing black masses of conidia. *Conidia* fusoid, straight, 24–*25·9*–28 μ × 7–*7·6*–8(9) μ; coloured cells umber, the 2 upper a shade darker, guttulate, 16–*17·6*–19 μ long; apical cell conoid or long conoid, bearing 3, rarely 2, rather stout, divergent spathulate setulae 13–*21·7*–27(28) μ long; basal cell conoid with a short pedicel 3–*4·1*–7 μ long.

On leaves of *Borassus aethiopum* Mart., Gold Coast (Colony); 2 June 1949 (BR.) (I.M.I. 37942*b*) (Gold Coast Myc. Herb. 123*b*).

(*b*) *Macules* indefinite. *Acervuli* very small, punctiform, producing globular masses of black conidia. *Conidia* fusoid, 25–*27·1*–31 μ × 5–*6*–7 μ; coloured cells umber with a greenish tinge, guttulate, the middle cell sometimes the smallest; end cells hyaline, the upper long conoid, bearing 3, sometimes 2, spreading spathulate setulae 14–*22·1*–32 μ long, the basal conoid with a pedicel 3–*5·8*–10 μ long.

On senescent leaves of ? *Dioscorea* sp.; Tafo, Gold Coast (Colony); 16 June 1949 (BR.) (I.M.I. 45126).

Subsection: VERSICOLORES.

Group: Fusiformes. Subgroup: Non-spathulatae

Pestalotiopsis maculiformans (Guba et Zeller) Steyaert, in *Bull. Jard. bot. Brux.* **19**, 329, 1949

Pestalotia maculiformans Guba & Zeller, in *Mycologia*, **24**, 370, 1932

Macules round or irregular, 0·25–2·5 cm. diameter, light brown. *Acervuli* hypophyllous, circular, scattered, producing black conidia adhering to leaf surface. *Conidia* fusoid, 19–*23·75*–28 μ × 6–*7·8*–9 μ; 3 middle cells 13–*15·8*–19 μ long, the 2 upper translucent fuliginous-brown, the lower yellowish brown; end cells hyaline, the upper conoid bearing 3, sometimes 2 or 4 spreading setulae 7–*16·6*–22 μ long, the basal with a pedicel 2–*4·3*–7 μ long.

On living leaves of *Landolphia owariensis* Pal. Beauv.; Aburi, Gold Coast (Colony); 24 May 1949 (BR.) (I.M.I. 44410*b*).

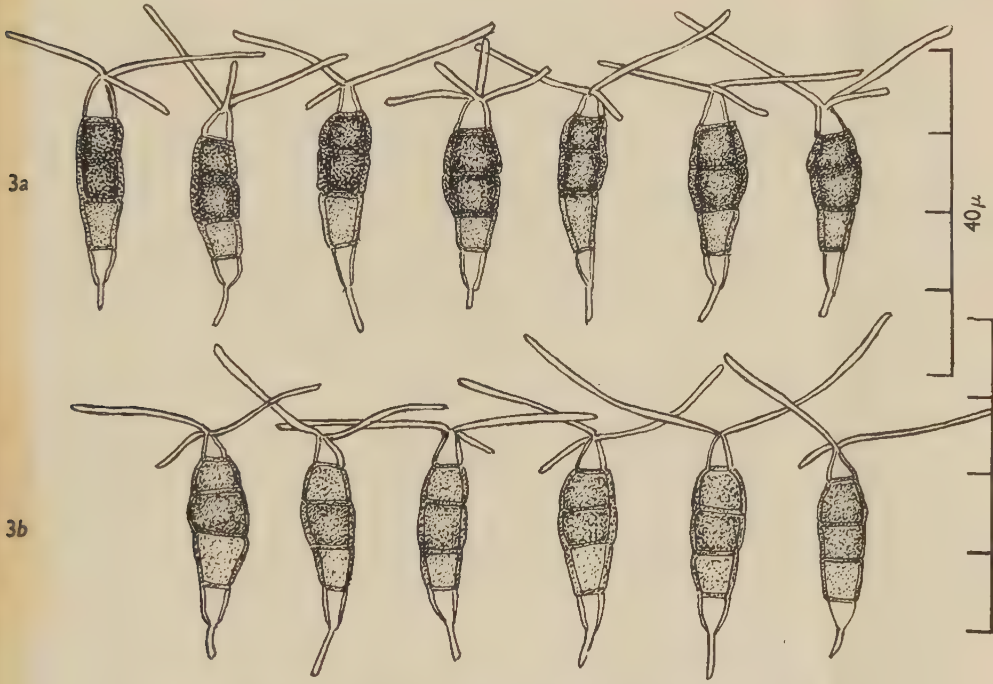

Fig. 3. *P. angusta* Steyaert: conidia; *a*, on *Borassus aethiopum* (I.M.I. 37920*b*); *b*, on *Bridelia ferruginea* (I.M.I. 42257); × 1000.

Pestalotiopsis angusta Steyaert sp.nov.

(*a*) *Maculae* diffusae. *Acervuli* hypophylli, rotundati vel elongati, minuti, primo epidermide tecti demum rotunditer fissi, plus minusve in lineam dispositi. *Conidia* fusoidea, recta, 4-septata, (20)21–*24·2*–27 μ × 6–*6·5*–7(8) μ; cellulae 3 mediae (13)14–*15·3*–*17*(18) μ longe, ad septa leniter constrictae, versicolores, 2 superiore umbrinae, inferior olivacea; cellulae extimae hyalinae, apicalis conoides 3, interdum 2 vel 4, setulas patulas (9)12–*17·5*–24 gerens, basalis conoides in pedicello 3–*4·2*–5(6) desinens. (Fig. 3*a*).

On living leaves of *Borassus aethiopum* Mart.; Techiman, Gold Coast (Ashanti); 6 April 1949 (BR., holotype) (I.M.I. 37920*b*, isotype).

(*b*) *Macules* diffuse, affecting large surfaces of leaf or internervular, greyish above, brown below. *Acervuli* amphigenous, circular, conical, small, sporulating more abundantly on the underside of leaf. *Conidia* fusoid, straight, 22–*24·6*–27 μ × 6–*7·2*–8 μ; middle cells 15–*15·9*–17 μ long, slightly constricted at the septa, versicolores, the 2 upper umber, the lower olivaceous; end cells hyaline, the apical conoid bearing 3, sometimes 2, divergent straight setulae 15–*20*–22(28) μ long, basal cell with a pedicel 3–*4·2*–5(6) μ long. (Fig. 3*b*).

On leaves of *Bridelia ferruginea* Benth.; Hohoe, Togoland; 28 May 1949 (BR.) (I.M.I. 42257) (Gold Coast Myc. Herb. 727*a*).

This species is close to *P. foedans* (Sacc. & Ell.) Steyaert but the conidia are longer with longer setulae and the two upper of the middle cells darker.

Pestalotiopsis aquatica (Ell. et Ev.) Steyaert, in *Bull. Jard. bot. Brux.* **19**, 334, 1949

Pestalotia aquatica Ell. & Ev., in *J. Mycol.* **5**, 157, 1889

(*a*) *Macules* irregular with greyish edge, otherwise brown above and below. *Acervuli* mostly epiphyllous, circular, flat, *c.* 300 μ in diameter, producing adpressed masses of black conidia. *Conidia* fusoid, 20–*23*–26 μ × 6–*6·8*–8 μ; middle cells 14–*15·1*–17 μ long, the 2 upper umber-fuliginous, the lowest olivaceous, end cells hyaline, the apical short conoid, sometimes subcylindrical, bearing 3, sometimes 2 or 4, widely divergent setulae 8–*12·5*–18 μ long; basal cell conoid, sometimes subspherical with a pedicel 2–*3·2*–5 μ long.

On living leaves of *Drypetes* sp.; Elmina, Gold Coast (Colony); 7 May 1949 (BR.) (I.M.I. 42111 *b*).

(*b*) *Macules* brown, irregular, with a sharply defined edge thinly marginated in dark brown. *Acervuli* amphigenous, very small, punctiform. *Conidia* fusoid-clavate, 18–*21·1*–25 μ × 6–*7·9*–9 μ; coloured cells 12–*14·3*–16 μ long the 2 upper translucent fuliginous brown, the lowest fulvous; end cells hyaline, the apical conoid bearing 3, or sometimes 2, spreading setulae (8)13–*17·2*–24 μ long, basal cell conoid with a pedicel 0–*3·1*–4 μ long.

On living leaves of ? *Harungana paniculata* Lam.; near Nsuaem, Gold Coast (Colony); 8 May 1949 (BR.) (I.M.I. 45096).

(*c*) *Macules* very irregular, reddish with a well-defined margin maroon-brown. *Acervuli* amphigenous, circular, about 100–150 μ in diameter, producing globular masses of conidia. *Conidia* fusoid-clavate 18–*19·5*–22 μ × 7–*7·8*–8 μ; coloured cells versicoloured, somewhat constricted at the septa, 12–*13*–17 μ long, the 2 upper fuliginous brown; end cells hyaline, the upper conoid bearing 3, sometimes 2 setulae, 9–*13·2*–21 μ long, the basal conoid to short conoid with a pedicel 0–*1·8*–4 μ long.

On living leaves of *Trachylobium verrucosum* (Gaertn.) Oliv. (Mr J. P. M. Brenan states: 'I think this is *T. verrucosum* (Gaertn.) Oliv. an introduced tree from East Africa'. Mr Hughes stated that this tree was growing in the Botanic Gardens at Aburi which would explain its presence in West Africa); Aburi, Gold Coast (Colony) 3 May 1949 (BR.) (I.M.I. 45097) (Gold Coast Myc. Herb. 928).

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CANESCIN, AN ANTIBIOTIC PRODUCED BY *PENICILLIUM CANESCENS*

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Canescin is an antibiotic produced by some strains of *Penicillium canescens* Sopp. A variety of media are suitable for its production, the main requirements being that the pH shall not rise above pH 6·5 during the course of the fermentation. It is extracted from active culture filtrates with chloroform in yields of 30–110 mg./l. Pure canescin is moderately antifungal, inhibiting germination of *Botrytis allii* spores at 12·5 μg./ml. It is only slightly bacteriostatic. It is unstable in aqueous solution; a solution in buffer at pH 3·5 loses 87·5% of its activity in 24 hr. at 25° C. and the loss in activity is even greater at higher pH. Canescin is sparingly soluble in water and common organic solvents but readily soluble in bicarbonate solution. It gives a purple colour with ferric chloride in ethanol solution. Analysis indicates the molecular formula $C_{15}H_{14}O_7$.

Introduction

Penicillium canescens Sopp may be regarded as a soil organism. Smith (1946) considered it to be commonly found in soil; Raper & Thom (1949) state that it is only occasionally found in soil, though widely distributed. It has also been recorded from soil by Sopp (1912) in Norway, by Dale in England in 1912 (quoted by Thom, 1930), by LeClerg & Smith (1928) from Colorado, by Bisby, James & Timonin (1933) in Canada and by Warcup (1951) from Breckland soils in England. We have isolated it from soils from a limestone cave; it is worth noting that in an extensive series of isolations from soils of the Bagshot sand type, we have never once encountered this species.

Production of antibacterial substances by *P. canescens* has been reported by Wilkins & Harris (1944) though no material was isolated. From our strains of this mould we have obtained a substance with moderate antifungal but little antibacterial activity.

Materials and methods

Methods of culture and assay of antibiotic activity were as previously described (Curtis, Hemming & Unwin, 1951). All cultures were incubated at 25° C.

Several isolates which we attribute to *P. canescens* were obtained from soil from a limestone cave at Ogof Ffynnon Ddu, Brecon, in 1948. On Czapek agar growth is rather restricted; the colonies are floccose, raised and contorted in the centre and radially folded; there is a broad (5 mm.) white growing edge surrounding a sage green sporulating area which shows traces of zonation; the reverse is at first yellow, becoming deep orange and finally orange-brown. Growth on corn-steep agar is similar but the colonies spread more rapidly. On malt-extract agar the colonies are looser in texture with a rather fimbriate edge. They are dull green in colour, with a colourless or light brown reverse. The conidiophores are divaricate bearing tangled or loosely parallel chains of conidia; they are mostly long (*c.* 500 μ), with rough walls and bear a number of terminal branches and metulae 10–50 μ long; each metula bears groups of four to eight phialides 7–9 μ long; the conidia are globose, 2·5–3·5 μ in diameter, with rough walls. This agrees with the description of *P. canescens* given by Raper & Thom (1949) in every respect except that our strains showed no tendency to produce conidial chains in columns.

We have been able to compare these isolates with one obtained by Warcup (1951) from Breckland soils—his no. B108. This is a very similar but not identical organism, being more restricted in growth; it is biochemically different from our strains and will be the subject of a further publication.

RESULTS

Antagonism in agar culture

Of four isolates tested, none showed any antagonism to *Candida albicans*, *Staphylococcus aureus*, *Salmonella typhi* or *Bacterium coli*.

Production of antibiotically active culture filtrates on liquid media

The work described below refers to results with one strain only, no. 880 in our collection. Some experiments have been repeated with other isolates from the same source with substantially similar results.

In preliminary experiments, on Czapek-Dox and Raulin-Thom media containing 5 % crude glucose in 30 ml. lots in 100 ml. flasks, titres of the order of 32 B.A. units/ml. were obtained in 10–14 days. The culture filtrates showed no antibacterial activity. The results of a more detailed experiment with these two media, with several glucose levels, are shown in Table 1. In this case the mould was grown on 250 ml. lots of media in 'Glaxo' culture vessels.

Under these conditions culture filtrates showed both antifungal and antibacterial activity. High antifungal titres were not produced on media containing 2·5 % glucose, and the slight activity seen early in the fermentation was not maintained. Maximum titres on 5 % glucose and 10 % glucose media did not differ significantly, though on the 10 % glucose media high activity was maintained over a longer period. It appears likely from the pH data that the substance or substances responsible for antifungal activity may be stable only at low pH; in every case as the pH of the culture filtrate reached about 7·0 the antifungal activity disappeared completely.

Antibacterial activity followed roughly the same course as antifungal activity in so far as it too disappeared as the pH of the culture filtrate rose. There were certain differences in the times at which maximum activity of the two types was reached, suggesting that the observed antifungal and antibacterial activity may not have been the effect of production of a single antibiotic.

There was little to choose between the Czapek medium (nitrate nitrogen source) and the Raulin-Thom medium (ammonia nitrogen source); further experiments, not reported in detail here, confirmed that the nitrogen source was not critical and that antibiotic production proceeded well in any medium supporting good growth in which pH did not rapidly reach a high level.

Table 1. *Effect of glucose concentration on development of antifungal and antibacterial activity and on* pH *drift*

	Days growth								
Medium	0	3	7	10	14	17	21	24	28
	B.A. units/ml.								
Czapek-Dox, 2·5% glucose	—	—	8	8	8	4	—	—	—
Czapek-Dox, 5·0% glucose	—	—	16	32	64	32	32	64	16
Czapek-Dox, 10·0% glucose	—	—	16	32	64	128	64	64	64
Raulin-Thom, 2·5% glucose	—	—	8	—	—	—	—	—	—
Raulin-Thom, 5·0% glucose	—	—	16	64	16	2	—	—	—
Raulin-Thom, 10·0% glucose	—	—	16	16	64	64	64	64	32
	Diameter of inhibition zone in *B. subtilis* assay								
Czapek-Dox, 2·5% glucose	—	—	11	—	—	—	—	—	—
Czapek-Dox, 5·0% glucose	—	—	8	13	14	12	10	11	—
Czapek-Dox, 10·0% glucose	—	—	15	18	19	17	17	18	17
Raulin-Thom, 2·5% glucose	—	—	—	—	10	—	—	—	—
Raulin-Thom, 5·0% glucose	—	—	16	11	—	—	—	—	—
Raulin-Thom, 10·0% glucose	—	—	18	18	18	15	14	14	13
	pH								
Czapek-Dox, 2·5% glucose	5·2	5·8	5·6	6·1	5·9	7·2	8·2	8·5	8·5
Czapek-Dox, 5·0% glucose	5·8	6·1	5·6	5·4	5·0	5·5	5·9	6·3	6·7
Czapek-Dox, 10·0% glucose	5·8	6·1	5·7	5·2	5·5	5·2	4·9	4·9	4·8
Raulin-Thom, 2·5% glucose	5·7	4·6	6·5	7·8	8·2	8·4	8·5	8·7	8·6
Raulin-Thom, 5·0% glucose	5·6	4·8	3·7	5·1	7·0	7·6	8·2	8·3	8·3
Raulin-Thom, 10·0% glucose	6·1	4·4	3·8	4·3	4·7	4·6	5·5	5·5	5·8

Isolation and characterization of canescin

The mould was grown for 13–15 days at 25° C. in earthenware vessels on a Czapek medium containing 5% crude glucose. The culture filtrate was adjusted to pH 3·5 with hydrochloric acid and extracted twice with one-fifth its volume of chloroform. The extracts were evaporated to dryness and the residue crystallized from a little ethanol. This material, obtained in yields varying from 30 to 110 mg./l. in different batches, we propose to call canescin.

Canescin may be purified by repeated crystallization from ethanol and glacial acetic acid. It forms clusters of colourless needles (from ethanol), m.p. 201–202° C. (decomp.). It does not contain nitrogen, halogen, sulphur or phosphorus. It is only sparingly soluble in the common organic solvents and in water, but readily dissolves, with effervescence, in cold sodium hydrogen carbonate solution to give a red solution, the colour of which soon fades on storage. In ethanol solution, it gives a purple colour with ferric chloride. It does not reduce Fehling's solution. Analysis indicates that it has the molecular formula $C_{15}H_{14}O_7$ with one methoxyl group. (Found: C, 58·5, 58·5; H, 4·6, 4·8; OMe, 10·2, 10·3; equivalent (by titration to phenolphthalein) 282. $C_{15}H_{14}O_7$ requires: C, 58·8; H, 4·6; OMe, 10·1; equivalent, 306 (monobasic acid).) This formula is supported by analysis of a *monomethyl derivative*, m.p. 159°, $[\alpha]_D^{20°} + 20°$ (*c*, 1·0 in chloroform) which it forms on treatment with ethereal diazomethane. (Found: C, 60·1; H, 5·3; OMe, 19·5; mol. wt. (Rast), 279. $C_{16}H_{16}O_7$ requires: C, 60·0; H, 5·0; OMe(2), 19·4; mol. wt., 320.)

Antibiotic activity of canescin

The minimum concentration of canescin in Weindling medium required to reduce germination of spores of a number of moulds to 5 % or less, is shown in Table 2. Canescin is not highly active. Concentrations (μg./ml.) of other antifungal antibiotics required to have a comparable effect on *Botrytis allii* are: albidin, 0·2; gliotoxin, 4·0; gladiolic acid, 8·0; glutinosin, 0·8; humicolin, 1·2; rubrogliocladin, 50·0; viridin, 0·01.

Table 2. *Fungistatic activity of canescin. Least concentration* (*μg./ml.*) *reducing germination to* 5 % *in Weindling medium* (pH 3·5)

Absidia glauca Hagem	> 100·0	*P. expansum* Link	50·0
Aspergillus niger van Tiegh.	> 100·0	*P. gladioli* McCull. & Thom	> 100·0
A. tamarii Kita	> 100·0	*P. stoloniferum* Thom	12·5
Botrytis allii Munn.	12·5	*Stachybotrys atra* Corda	12·5
Fusarium caeruleum (Lib.) Sacc.	25·0	*Stemphylium* sp.	100·0
F. graminearum Schwabe	25·0	*Thamnidium elegans* Link	25·0
Penicillium brevicompactum Dierckx	12·5	*Trichoderma viride* Pers. ex Fries	> 100·0
P. digitatum Sacc.	50·0		

Canescin is only slightly antibacterial. Growth of *Bacterium coli* and *Salmonella typhi* in broth at pH 5·5 or 7·0 is not inhibited by 100μg./ml. Growth of *Staphylococcus aureus* is inhibited by 100 μg./ml. at pH 5·5 but not at pH 7·0. Two strains of *Bacillus subtilis* were inhibited at 100 and 25 μg./ml. at pH 5·5 but not by 100 μg./ml. at pH 7·0. No inhibition zone was produced in the *B. subtilis* cylinder plate assay by a 100 μg./ml. solution of canescin; the antibacterial activity recorded in Table 1 must therefore have been largely due to some other substance. The antifungal activity recorded in culture filtrates from *Penicillium canescens* can usually be more or less completely accounted for by the yield of canescin.

Stability of aqueous solutions of canescin

Canescin was dissolved in McIlvaine buffer in a range of pH from 3·5 to 7·5 and assayed after 24 hr. at 25° C. In the range pH 3·5–5·0 the activity was reduced to 12·5 % of that of the original solution; at pH 6·0 and above almost all activity was lost. The explanation given earlier of the loss of activity in fermentations where the pH rises (see Table 1) is thus probably correct. Canescin is a very unstable antibiotic, comparable with albidin in this respect (Curtis *et al.* 1951).

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VARIATION IN A STRAIN OF *PHYTOPHTHORA CACTORUM*

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(With Plates 14 and 15)

A number of variants derived from a zoospore isolate of *Phytophthora cactorum* are described, and their colony characteristics, morphology of vegetative hyphae, and size, appearance, and frequency of reproductive organs are compared. The variants have been maintained to some extent on germination of single zoospores and oospores. While no means has been found of controlling these changes by altering environmental conditions, there is a connexion between variation and ageing, reversion to the original form having occurred in old cultures of all the variants.

Introduction

In a study of variation of a strain of *Phytophthora cactorum*, originally isolated from onion by Dr C. J. Hickman, thirty-one cultures started from single zoospores were isolated and examined on plates of maize-extract agar. One colony differed from the rest in its slower growth and more compact and less feathery appearance (Pl. 15, figs. 1 and 2). Zoospores isolated from it gave rise to cultures among which reversion and a third variant (Pl. 15, fig. 6) were recorded. With indication that a unizoospore culture could vary, further investigation was made of the range of natural variation of cultures derived from the slow-growing zoospore isolate, of the relative stability of the variants, and of the differences between them.

A detailed study of variation in the genus *Phytophthora* was made by Leonian (1925), who recorded a lack of stability in some isolates, in particular in *P. parasitica* var. *rhei*, of which five different forms were obtained, two of them changing readily one to the other or giving complex colonies. Attempts to control this fluctuation by manipulation of environmental factors and by repeated passage of the fungus through its host plant were unsuccessful (Leonian, 1926). In 1934 he applied the term 'dissociation' to this phenomenon, defining it as 'an emergence or submergence of different potentialities of the same organism'. Under the influence of certain chemicals and over a temperature range differences among the dissociants of *P. parasitica* var. *rhei* were demonstrated comparable with those used to make specific distinctions within the genus.

Adaptive changes in virulence of strains of *P. infestans* have been reported by a number of workers, including Reddick & Mills (1938), Mills (1940), and de Bruyn (1951).

Although detailed accounts are available of the life history of *P. cactorum* (Blackwell & Waterhouse, 1931; Blackwell, 1943; Waterhouse, 1931), no records have been found of investigation of variation in this species.

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METHODS

No attempt was made to induce variation, some of the variants being collected among zoospore isolates, while others arose as sectors on agar plates. Colonies are described as they appeared when grown at 20° C. on plates of clear maize-extract agar of the following composition: ground-maize meal 50 g., agar 20 g., water 1000 ml. Maize powder extracted with water at 60° C. for one hour, filtered; agar added; medium filtered again. 15 ml. of medium were used for each 9 cm. dish and inoculum was taken with a cork-borer, 0·4 cm. in diameter, at about 0·5 cm. from the edge of a growing colony. An experiment showed that little or no variation arose from the use of different batches of medium, so that the production of the very distinct forms here described cannot be attributed to any lack of uniformity of the medium.

In preparing zoospore isolates a fragment of a culture on bean-extract agar (ground seeds of Canadian Wonder dwarf bean 30 g. were mixed with 1000 ml. water and boiled for 5 min., filtered; 20 g. agar added) on which sporangia were produced in abundance, was transferred to a drop of sterile distilled water. Zoospores, emerging after a few minutes, were streaked on agar plates and cut out singly when germinated. Occasional large multiple zoospores with more than one pair of flagella visible, and presumably more than one nucleus, were avoided.

The procedure used in germinating oospores was similar to that described by Blackwell (1943). Slant cultures kept under laboratory conditions for 6 months were transferred to a refrigerator at 5° C. for a week or longer. A small piece of a culture was then teased out in a drop of sterile tap water and the larger portions of agar and mycelium were removed, leaving a suspension of detached oogonia and smaller fragments of hypha, which was streaked over an agar plate. During the following 2 days, many germinated oospores could be isolated.

EXPERIMENTAL RESULTS

Colony characters of the variants

In the following descriptions the term 'arbuscular' is used to denote a gnarled, knobbly appearance of the hyphae (Pl. 14, fig. 2), contrasting with their usual fine, even character (Pl. 14, fig. 1). Representative letters are given as a symbol of each variant.

RS (Pl. 15, fig. 1)

A regular, slow-growing colony developed from one of the thirty-one zoospores first isolated, and from which every other form was ultimately derived. *Hyphae:* non-arbuscular; *oogonia:* abundant, appearing after 4 days; *sporangia:* few, after about 10 days.

RF (Pl. 15, fig. 2)

A regular, fast-growing colony. The form in which the fungus was first observed, it occurred in thirty of the thirty-one zoospore isolates and was reisolated later from the odd one. *Hyphae:* highly branched but not arbuscular; *oogonia:* very abundant, after 3 days; *sporangia:* few, after 7–8 days.

Z (Pl. 15, fig. 3)

A zoned colony derived from a sector in a culture of RF. In successive subcultures fewer and wider zones were developed until reversion to the RF condition was complete. *Hyphae:* non-arbuscular except at the margins of the zones, where irregular clusters of swollen branches developed, with a pause for a day or two in radial extension; *oogonia:* when first isolated very few and only after 10 days more numerous and formed earlier in later subcultures; *sporangia:* very few in early cultures, after several weeks.

AF (Pl. 15, fig. 4)

An arbuscular colony of fast growth, frequently observed on subculturing from RF, and reverting readily to the regular condition. *Hyphae:* arbuscular; *oogonia:* varied in number but were not abundant, often appearing distorted in shape; *sporangia:* few.

AS (Pl. 15, fig. 5)

An arbuscular, slow-growing colony derived from RS, similar in appearance to AF.

T (Pl. 15, fig. 6)

A transitional form which occurred among zoospore isolates and mycelial transfers from RS, and from which subcultures were generally of the AS type. At about 5 days old the colony appeared quite regular except for a central slightly denser zone. Growth at the margin then became sparse and irregular with arbuscular hyphae, while the central area thickened, developing abundant oogonia and sporangia and a mat of aerial mycelium. Frequently, in older cultures, the radiating arms of arbuscular mycelium became joined by a web of finer hyphae bearing numerous sporangia, which eventually filled the plate.

Isp (Pl. 15, fig. 8)

An irregular type of colony with numerous sporangia and few oogonia, cultured from a number of the slow-growing forms (Pl. 15, fig. 7). After some regular growth, swollen branches were developed round the edge of the colony, from which irregularly rounded patches of mycelium were formed, these in turn becoming arbuscular. *Hyphae:* fairly even except at the margins of the patches; *oogonia:* few, large; *sporangia:* very numerous, large, sometimes starting to develop before the oogonia.

Rsp (Pl. 15, fig. 9)

Obtained on mycelial transfer from Isp, which it resembled in producing sporangia in greater abundance than oogonia, but from which it differed in its more regular growth. The sporangia of this variant, which were large, germinated by zoospore liberation less readily than did those of any other variant.

SS (Pl. 15, figs. 10–12)

A variant of exceptionally slow growth, collected among zoospore isolates from AS. An extremely unstable form, it was maintained only by subculturing early in the development of a colony, which invariably changed round the edge as illustrated to give other forms. *Hyphae:* hardly arbuscular, but sinuous in outline, highly branched and growing together very compactly; *oogonia:* numerous; *sporangia:* none until about 14 days old, then becoming numerous.

RFs-s

A regular colony, similar in appearance to RF but of slightly higher growth rate and producing smaller spores. The type originated as rapidly growing sectors in plates inoculated from old cultures of RF and Isp. *Hyphae:* very fine, branching evenly; *oogonia:* abundant, small; *sporangia:* few, small.

Variation in size of reproductive organs

In comparisons of the size of reproductive organs measurements were made of oogonia in 6-day-old colonies on maize-extract agar, and of sporangia in mycelium grown in liquid bean extract and transferred to distilled water for 2 days. Differences significant at the 1 % level were found between the RF type and each of the three other variants measured for oogonial diameter and sporangial length (Table 1), and between RF and Isp for shape of sporangia as defined by the ratio length/breadth.

Table 1. *Variation in oogonial diameter and in sporangial length*

Variant	Mean of 100 measurements in μ ± standard error of mean	Difference in microns from RF
Oogonial diameter		
RF	30·32 ± 0·50	—
AS	32·11 ± 0·32	1·79 ± 0·59
Isp	34·13 ± 0·22	3·81 ± 0·55
RFs-s	22·85 ± 0·12	7·47 ± 0·51
Sporangial length		
RF	48·03 ± 0·36	—
AS	42·53 ± 1·06	5·50 ± 1·12
Isp	53·60 ± 0·33	5·57 ± 0·49
RFs-s	35·47 ± 0·31	12·56 ± 0·48

Spore germination

Zoospores and oospores were germinated and isolated to determine to what extent the characters of the variants were maintained. On zoospore isolation, the variants RF, SS, Isp and RFs-s were fairly stable. All zoospores from the zoned culture Z produced colonies of the RF type, while from the arbuscular form AS, cultures of RS, T and SS were obtained.

Germination of twenty oospores of each of the four variants RF, AS, Isp and RFs-s showed that their characters could be carried over and reproduced. At the same time, oospore isolation revealed evidence of further variation and reversion, one oospore from AS giving an Isp culture, one from Isp developing into AS, and 18 from Isp and four from Rs-s producing the RF type.

Effect of environmental factors

Experiments were designed to reveal possible control of changes between the variants. There appeared to be no evidence of any modification of a medium by one variant to induce a lasting change on another form grown on it later. Other tests confirmed the stability of the three variants RF, AS and Rsp over a wide range of controlled conditions, including different concentrations of several media, various temperature levels and alternation of high and low temperatures, and a range of carbon/nitrogen ratios. Inoculation of apples (var. Newton Wonder) and re-isolation had no permanent effect on colony characters, but demonstrated differences in pathogenicity, the variant AS being non-pathogenic.

Ageing of cultures

Observation of reversion to the RF condition in old cultures of all the other variants led to further investigation of ageing as a factor in variation. By making a series of subcultures, the last after 6 weeks, from each of nine marked areas in a plate culture on maize-extract agar, a method similar to that of Brown (1926), variation could be detected in colonies showing no visible change, the proportion of unstable areas increasing with age. Thus from AS three of the nine samples after 2 weeks and six after 4 weeks gave colonies showing a tendency to form sectors of higher growth rate and more numerous sporangia. From Rsp reversion to RF occurred in one sample after 4 weeks and four after 6 weeks. RF showed no sign of instability apart from one colony of the AF type derived after 6 weeks.

Similar but more rapid changes were recorded using a method similar to that of Leonian for *Fusarium moniliforme* (1932) to demonstrate the potentialities of an inoculum disk on ageing. Three 0·4 cm. disks, cut from the edge of a growing colony of each variant on maize-extract agar, were transferred to plates of the same medium. Every 48 hours, over a period of 16 days, the same disks were removed to fresh plates and the series of colonies were examined. Cultures of the arbuscular type AF were grown from all three disks of RF after the fourth transfer. Sectoring colonies were produced by two AS disks in the seventh set of plates, and by three in the eighth set. Of the Rsp disks, one after the seventh transfer and all after the eighth were producing RF sectors.

In an attempt to determine the effect of different conditions during ageing on any tendency for variation, slope cultures of RF on various agar media were kept at 5 and 20° C., and under laboratory conditions in light and darkness. Plates inoculated from these slopes after 3, 6 and 12 months were examined for variants. Some colonies were of the arbuscular form AF, and some sectored to produce the small-spored variant RFs-s, but no correlation could be made between tendency for variation and the growth conditions.

DISCUSSION

In the present investigation one zoospore has given rise readily to a range of colony types of varying stability, differing in habit, growth rate and morphology of hyphae, in size, shape and frequency of reproductive organs, and in pathogenicity, though it is not known to what extent such changes occur among other zoospore isolates of this strain.

The nature of the variation remains obscure. Proof of gene mutation could be obtained only from breeding experiments, hardly possible in a homothallic form. Alternatively, the gradual nature and ready reversibility of some of the transformations could perhaps better be explained in terms of cytoplasmic change, for which, again, there is little hope of confirmation.

Increased variability in old cultures appears to be unconnected with any change in physiological conditions and can be regarded rather as the consequence of a longer period of time allowed for the establishment of new forms. Under ordinary conditions of culture variants may arise but never be detected because of competition with the parent part of the colony. Thus the true variability of such a fungus can be appreciated only by such methods as subculturing from different points in a plate culture, examining the potentialities of an inoculum disk, or isolating zoospores. As a means of acquiring a broader knowledge of *Phytophthora* species, such an investigation is important since there can be no doubt concerning the relationship of the different forms. Only in this way can the real taxonomic value of differences in such characters as frequency, size, and shape of reproductive organs, and pathogenicity of isolates be assessed.

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EXPLANATION OF PLATES 14 AND 15

PLATE 14

Fig. 1. Regular type of mycelium from colony RF. × 390.
Fig. 2. Arbuscular hyphae from IS. × 390.

PLATE 15

(all approximately × ½)

Fig. 1. RS, 8 days old.
Fig. 2. RF, 8 days old.
Fig. 3. Z, 18 days old.
Fig. 4. AF, 9 days old.
Fig. 5. AS, 14 days old.
Fig. 6. T, 14 days old.
Fig. 7. RS, sectoring to Isp.
Fig. 8. Isp, 15 days old.
Fig. 9. Rsp, 15 days old.
Figs. 10–12. SS, changing to other forms.

(*Accepted for publication* 29 *August* 1952)

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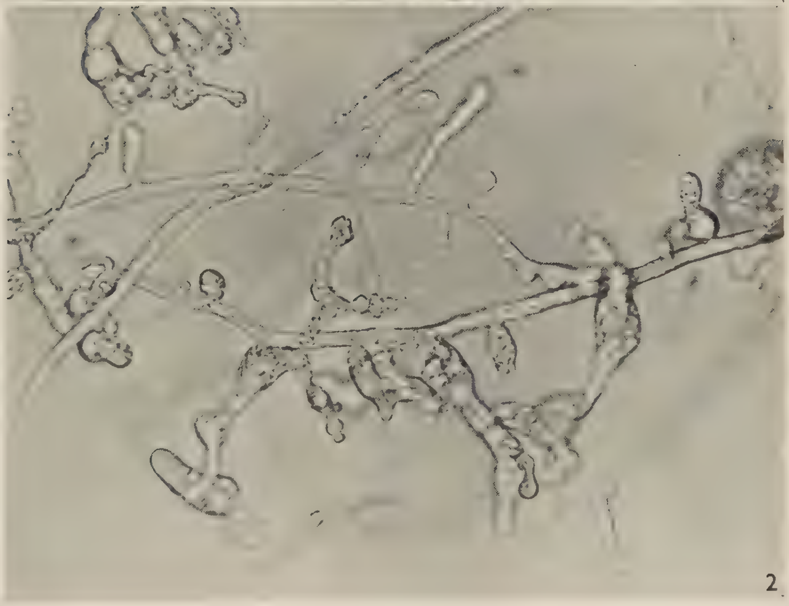

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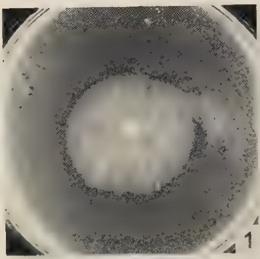
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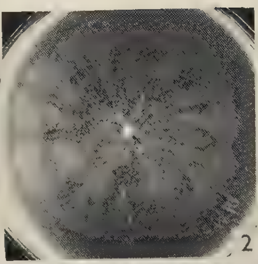
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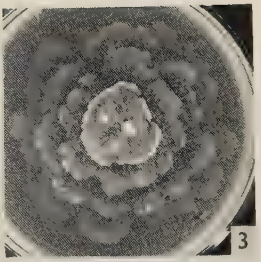
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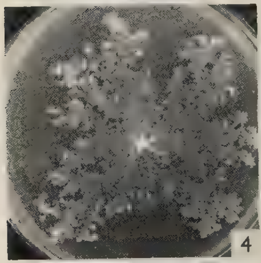
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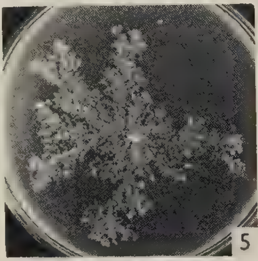
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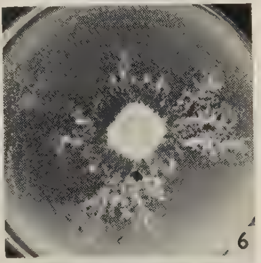
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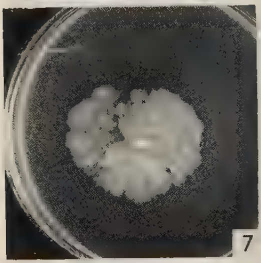
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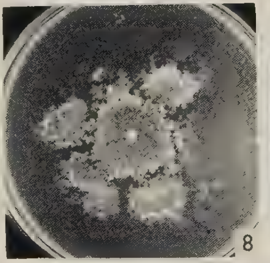
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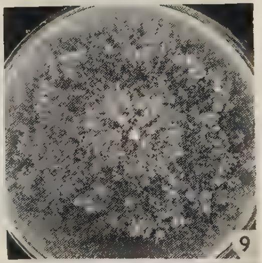
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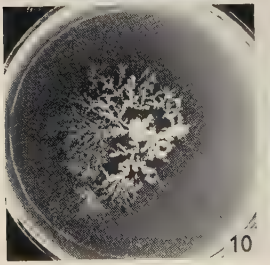
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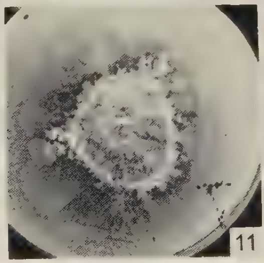
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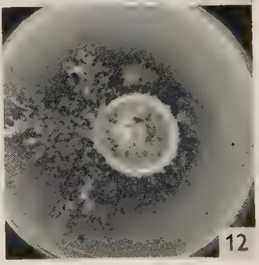
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OOSPORE PRODUCTION IN PAIRED CULTURES OF *PHYTOPHTHORA* SPECIES

By D. JEAN STAMPS*

Department of Botany, University of Birmingham

(With Plate 16)

Using a slide-culture technique examination of four strains of *Phytophthora cinnamomi* and five of *P. cryptogea*, growing in pairs, has shown that hybridization takes place, *P. cinnamomi* forming the antheridium and *P. cryptogea* the oogonium. Later, some of the *cinnamomi* isolates form non-hybrid oospores, apparently stimulated by conditions in the dual culture. A sixth *cryptogea* strain produces oospores when grown with members of either species.

INTRODUCTION

Numerous reports have been made of sexual reproduction in paired *Phytophthora* isolates belonging to the same or different species, which rarely, if ever, produce oospores in pure culture. The main subject of controversy has concerned the two alternative theories of oospore formation, whether, on the one hand, by the union of oogonia and antheridia borne on separate mycelia belonging either to different strains of a heterothallic species or to different species, or, on the other, by chemical stimulation of one or each member to produce both types of sexual organ. In support of the first view, direct observations of oogonial and antheridial connexions between different strains were made by Narasimhan (1930) in *P. arecae* with the aid of perforated mica strips to limit the number of hyphae concerned in reproduction; by Leonian (1931), who examined paired members of his *omnivora* group in clear malt agar; by Barrett (1948), who identified two isolates of *P. drechsleri* as male and female, respectively; and by Cohen (1950), who paired *P. palmivora* with *P. cinnamomi* on the cover-slip of a van Tieghem cell. Direct evidence of chemical stimulation was provided by Galloway (1936), who induced oospore formation in single cultures of *P. meadii* and *P. colocasiae*, not normally producing them, by addition to the medium of unheated filtrate from a paired culture.

The present investigation was aimed at examining this problem as it applied to *P. cryptogea* and *P. cinnamomi*, species already reported to produce oospores in mixed culture by Ashby (1929) and Barrett (1948).

ISOLATES

Hyphal tip cultures were prepared of six isolates of *P. cryptogea* and four of *P. cinnamomi*, numbered as follows: *P. cryptogea*—65, Lister Institute; 66, Baarn (Tucker); 67, Baarn (Pethybridge); 68, 69 and 70, Botany Department, University of Birmingham (Hickman, isolated from onion).

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P. cinnamomi—91, Baarn (Rands); 109, Baarn (Leonian, isolated from *Rhododendron*); 116, Commonwealth Mycological Institute (Pimentel, isolated from *Pseudotsuga*); 117, Commonwealth Mycological Institute (Pimentel, isolated from *Betula*).

All were typical for their species as regards hyphal characters, temperature relations, and morphology and size of sporangia and, where formed, of sexual organs.

Sexual reproduction in single cultures

Examination of 4-month-old cultures on oat agar slopes revealed a few oospores in the *cryptogea* strains 68 and 65 and in *cinnamomi* 109 but in none of the other isolates. Except for a few produced occasionally by *cryptogea* 69 none were found in plate cultures up to 6 weeks old on bean- and maize-extract agar, prepared as described by Stamps (1953). Transfer of sterile mycelium to M/100-potassium nitrate solution, as described by Tucker (1931) for *P. cinnamomi*, was effective in inducing sexual reproduction by isolates 109 and 117.

Paired cultures

Preliminary examination over a period of 4 weeks of all possible combinations of strains, paired on plates and slopes of maize- and bean-extract agar at 20° C., established that sexual organs were formed in abundance 3 days after the meeting of any *cinnamomi* colony with any *cryptogea* except strain 65. Oogonia were similar in size to those from single-strain *cryptogea* cultures, the average diameter of fifty measured from a 68 × 109 (*cryptogea* × *cinnamomi*) cross being 31·5 μ, compared with 32·3 and 38·7 μ recorded, respectively, for 68 and 109 separately. Later a few conspicuously larger oogonia with apparently two-celled antheridia, characteristic of *P. cinnamomi*, developed in paired cultures containing strains 109 or 117 (Pl. 16, fig. 1). None found in single cultures of the same age and grown under the same conditions.

Cryptogea 65 behaved unexpectedly in reproducing sexually when paired with any other isolate, though more readily with other *cryptogea* strains than with *cinnamomi*, the oogonia being similar in diameter (average 27·4 μ) in all crosses and absent from corresponding single cultures. Later attempts to identify antheridial and oogonial connexions in crosses with this strain were unsuccessful.

Preparation of slide cultures

Having established the behaviour of the different strains in mixed culture, various methods were tested for facilitating observation of oogonial and antheridial connexions. Eventually a technique was devised for pairing cultures in a thin layer of agar. Two minute pieces of inoculum, each containing only a few short hyphal tips, were cut from the edges of *cryptogea* and *cinnamomi* colonies growing on clear maize-extract agar and placed together on a sterile glass slide. A sterile cover-slip was pressed down over them so that the agar ran into a continuous thin layer, surrounding and separating the two groups of hyphae. Stacked in large

Petri dishes lined with moist filter-paper, the slides were incubated at 20° C. until the colonies grew together and sexual organs were formed, within 3–6 days.

Such preparations could be made permanent. With the cover-slip secured with cotton they were fixed by immersion for 24 hours in chrom-acetic acid, washed, and stained by soaking in 1 % aqueous solution of cotton blue until the dye had penetrated through the whole preparation. Following further washing they were sealed after drawing glycerine, to which a crystal of phenol had been added, under the cover-slip.

In this way both single and dual cultures of *cryptogea* and *cinnamomi* isolates were prepared and examined more intensively than had before been possible. The following observations were made:

(1) The hybrid nature was confirmed of the small *cryptogea*-like oospores formed immediately on pairing any of the *cinnamomi* isolates with *cryptogea* strains 66, 67, 68 and 70. In all cases where observation was possible, associated with a pair of reproductive organs were two hyphae which could be identified as belonging to opposite species. The distinction was easily observed because the knobbly, swollen *cinnamomi* hyphae contrasted clearly with the more even growth of the *cryptogea*. In crosses of *cryptogea* 69 with any *cinnamomi* some of the small oospores were hybrid and some pure *cryptogea*.

(2) In all cases of hybridization antheridia were developed by the *cinnamomi* strain and oogonia by the *cryptogea* as had been suspected from oogonial measurements (Pl. 16, figs. 2 and 3).

(3) Conditions of growth seemed favourable for the formation of sexual organs, a few of which were found in pure cultures of *cryptogea* 69 and the *cinnamomi* strains 109, 116 and 117.

(4) In dual cultures which included any of these three *cinnamomi* isolates, some pairs of sexual organs developed, of which both oogonium and antheridium could be identified as *cinnamomi* (Pl. 16, fig. 4). They appeared 4–5 days earlier than those in corresponding single cultures.

Discussion

Identification of the hyphal connexions of reproductive organs in paired cultures, using four strains of *P. cinnamomi* and five of *P. cryptogea*, has shown that hybridization occurs in the early history of a culture, *P. cinnamomi* forming the antheridium and *P. cryptogea* the oogonium. This differentiation is supported by the fact that oogonia in paired cultures are similar in size to those found in pure *cryptogea* cultures, an observation made by Ashby (1929), who, however, attributed it to stimulated reproduction of *P. cryptogea*. In contrast, Barrett (1948) identified his strains of *P. cinnamomi* as female and all but one of his *cryptogea* isolates as male.

In addition to hybridization, evidence was obtained of oospore formation due to chemical stimulation. The development of large oogonia and antheridia, both on *cinnamomi* hyphae, in some dual cultures appears to result from such stimulation, either from the *cryptogea* mycelium or as a result of the earlier formation of hybrid oospores, for none is found in single cultures of the same age growing under the same conditions. It is possible that some of the *cryptogea*-like oospores in *cryptogea-cinnamomi* cultures may be pure *cryptogea* since, under the experimental conditions, only the earliest sexual organs to be formed can be identified. The behaviour of *cryptogea* 65 in producing small *cryptogea*-like oospores, whether paired with *cinnamomi* or other *cryptogea* strains, can also best be explained in terms of chemical stimulation. Lack of direct support for the chemical stimulation theory for any pair in experiments with solid media, with unheated filtrates from single and dual liquid cultures, or by constantly exchanging a liquid medium between two colonies may indicate a need for a high local concentration of any possible stimulant before it becomes effective. It is emphasized that no generalizations should be made for the whole genus concerning oospores in mixed cultures, but that each pair of species should be examined separately, while any proof of hybridization obviously need not imply strict unisexuality of the strains concerned.

In a study of the taxonomy of the genus and its plant pathological aspect demonstration of the formation of hybrid oospores is significant. Their germination would be of even greater interest, particularly in view of the differences in general morphology, physiology and pathogenicity between *cinnamomi* and *cryptogea* isolates. Numerous attempts were made to germinate oospores from paired cultures up to 12 months old. None was successful, possibly because the oospores were not old enough, or conditions effective in breaking the dormancy period were not found, or because of failure of nuclear fusion.

I wish to express my thanks to Dr C. J. Hickman, under whose direction the work for both this and the preceding paper was carried out, to Dr Mary Gregg for much helpful advice, and to Miss G. M. Waterhouse, who kindly supplied some of the cultures and read the manuscripts.

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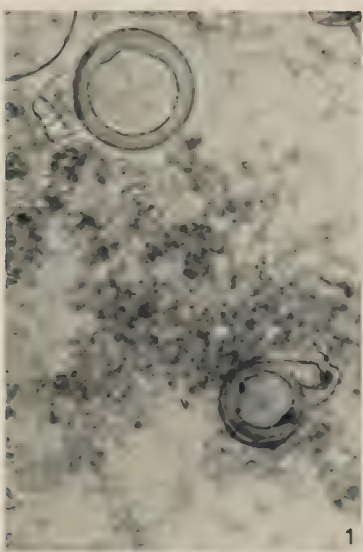
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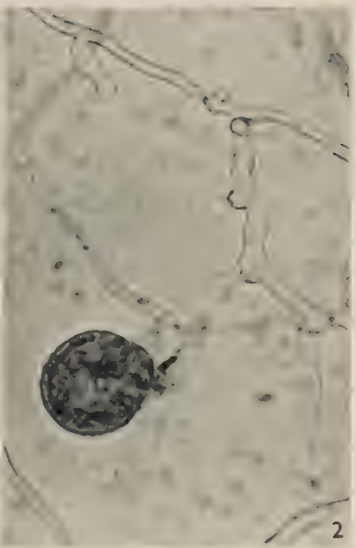
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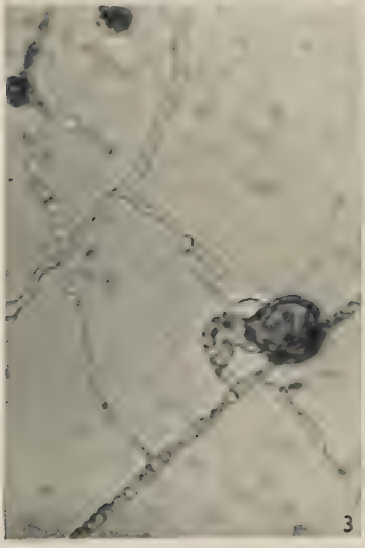
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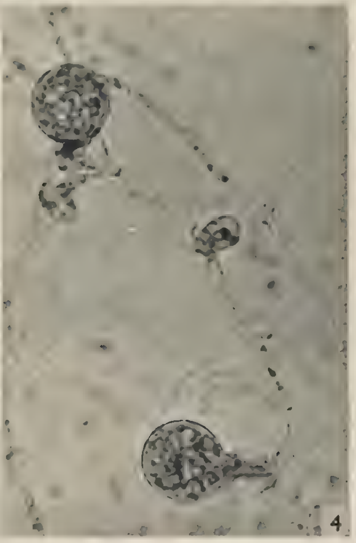
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EXPLANATION OF PLATE 16

Fig. 1. Large and small oospores in a dual culture of *Phytophthora cinnamomi* 117 and *P. cryptogea* 66. × 390.

Fig. 2. Sexual organs in a paired culture, with a hypha of *P. cryptogea* 66 connected with the oogonium and *P. cinnamomi* 109 forming the antheridium. The abnormally large size of the oogonium is due to the pressure of the cover-slip during drying and contraction of the agar. × 390.

Fig. 3. Sexual organs in a paired culture. Mycelium of *P. cinnamomi* 91, with characteristic swellings, is in the upper left-hand corner; *P. cryptogea* 69 is below, right. × 390.

Fig. 4. Early stage in the formation of hybrid (upper) and *cinnamomi* (lower) oospores. Both sexual organs of the lower pair are produced by *P. cinnamomi* 109; the hypha connected with the oogonium continues to form the antheridium of the upper pair, fertilizing a *cryptogea* 66 oogonium. × 390.

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THE INCIDENCE OF *CLADOSPORIUM HERBARUM* IN THE OUTDOOR AIR AT CARDIFF, 1949–50

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(With 3 Text-figures)

The most abundant mould caught on Petri plates exposed outdoors during 1949–50 was *Cladosporium herbarum*, the incidence of which (as summarized week by week) showed a marked seasonal rhythm with a maximum in summer and a minimum in winter. Incidence was markedly higher during the warm dry summer of 1949 than during the relatively cool wet summer of 1950. Counts of conidia caught on 24 hr. slides exposed at certain times simultaneously with the plates indicate a much lower average incidence. Possible causes of this discrepancy are discussed, and it is concluded that it is probably due to a higher incidence in the morning than the average for the whole 24 hr.

The earliest estimates of the incidence of *Cladosporium* in the air appear to have been made by Rostrup (1908). A survey of atmospheric moulds, including *Hormodendrum* (*Cladosporium*), was begun by Feinberg in 1935 (Feinberg & Little, 1936). More widespread surveys have been made by Morrow, Lowe & Prince (1942), and on behalf of a subcommittee of the American Academy of Allergy (Harris, 1950). Slide counts from eighty-eight centres in North America have been made by Durham (1946), who has also made counts on slides exposed in the U.S.A. from aeroplanes (Durham, 1942). High-level observations (using plates) have been made too by Pady, Kelly & Polunin (1948) in the Canadian Arctic. Other data have been contributed from the continent of Europe by Nilsby (1947, 1948, 1949), Rennerfelt (1947), Flensborg & Samsøe-Jensen (1948), and Vallery-Radot, Halpern, Secretain & Domari (1950).

In view of the probability that atmospheric moulds would prove to be significant as causes of allergic disease in Great Britain as they had already been shown to be in America a preliminary census of such moulds at Cardiff was made by exposing Petri plates at intervals throughout the year 1948 (Hyde & Williams, 1949): under the conditions of sampling employed the most abundant type to appear was *C. herbarum*. The census was continued during the whole of 1949 and 1950, and during certain periods the plate counts of moulds were supplemented by slide counts of *Cladosporium* conidia. The present communication relates to *Cladosporium* only: the other moulds which appeared and were identified are only referred to incidentally.

THE PETRI PLATE SURVEY

Method

Two or more 9 cm. plates of Sabouraud's agar or other suitable medium were exposed for 10 min. at 10 a.m. on 6 days each week during the whole 2 years. The plates were placed for this purpose on the lower disk of our standard apparatus (Hyde, 1950), which was situated on the flat roof of the operating theatre at Llandough Hospital near Cardiff. Both situation and mode of exposure were chosen deliberately so as to ensure that sampling was made from outdoor air, not from air coming from the inside of the building. This had been intended to apply to the 1948 census, but owing to a misunderstanding the plates during the greater part of that season were exposed on an outdoor window-sill and the significance of the results obtained was (and must remain) ambiguous.

Two Sabouraud's agar (maltose-peptone, prepared in the laboratory) plates were exposed for 10 min. on each occasion throughout except from 5 April to 16 August 1949 (inclusive) when one was replaced by potato-dextrose agar. Additional plates were exposed simultaneously as follows: two 5 min., Sabouraud's agar plates in succession: 18 August to 4 November 1949; ready prepared Sabouraud (Oxoid brand, code no. CM41, glucose-peptone), one plate, 17 February to 1 March and 20 June to 30 December 1950. It was concluded from consideration of the catches that the various media gave closely similar results. All the counts have therefore been aggregated day by day and expressed in terms of one 10 min. plate per day.

After exposure the plates were incubated at room temperature for 5 days, and all colonies which developed in that time were determined generically as far as possible and counted. *Cladosporium* colonies developed very rapidly and, during the peak of the season, in such numbers that they appeared largely to inhibit the development of other types: for this reason we think that while the counts of *Cladosporium* colonies are probably a measure of the incidence of conidia (or rather of conidial units of dispersal, see below) on our plates, the counts of other genera probably have not always the same significance. Over the whole of the 2 years *Cladosporium* made up 68 % of the plate counts; at the peak of the season this figure rose as high as 96 %.

The *Cladosporium* colonies practically all fell into two groups: (*a*) non-woolly, with small conidia averaging $5 \times 3\,\mu$; (*b*) woolly, with large conidia averaging $17{\cdot}6 \times 8{\cdot}9\,\mu$ (data from Mr Merfyn Richards). During 1950, 12·7 % of all the *Cladosporium* colonies on our plates were of the large-spored kind. Following Brooks & Hansford (1923), who also distinguished strains with woolly and non-woolly colonies, we have regarded both (*a*) and (*b*) as forms of *C. herbarum.*

The annual cycle

The weekly totals, both of all mould colonies (excluding yeasts) and of *Cladosporium*, from 3 January 1949 to 30 December 1950 are set out in Figs. 1 and 2. The counts of *Cladosporium* are seen to fall into a similar pattern each year: in both they oscillated about a low figure for nine months and rose to relatively high values during a period of about 3 months in the summer. There were, however, marked differences between the two seasons. The total count for 1949 (4777 expressed as on one daily 10 min. plate on 6 days of every week) was 52 % higher than that for 1950 (3127); the peak of the 1949 season (525 per 6-day week) was very distinctly higher than that for 1950 (414); and occurred distinctly later (1–6 August 1949 as against 10–15 July 1950); and in fact the *Cladosporium* 'season' as a whole (if defined arbitrarily as the period during which 120 colonies were registered per week) fell distinctly later in 1949 (13 weeks, 27 June–24 September than in 1950 (12 weeks, 22 May–12 August).

*Relations with temperature**

The differences in the count correspond with differences in the temperature record. The mean average temperature for June 1949 was 3·1° F. above the 58-year average, and that for June 1950 was still higher (+3·8° F.). The mean average temperatures for July, August and September 1949 were also higher than normal (+4·5, +3·0 and +5·5° F. respectively); those for the corresponding months in 1950 were below normal (−2·8, −3·0 and −0·6° F.).

In 1949 the weekly mean temperature (Fig. 1) remained below 60° F. until the week ending 11 June and the *Cladosporium* count too remained much below 100. A rise to 62° F. for 12–18 June was followed by a rise in the count to over 100. The mean temperature remained above 60° F. until 31 July–6 August and counts kept up (300–525). A fall in temperature during 7–13 August was reflected in a drop in the next week's count to only just above 100, but a further rise in the temperature during 21–27 August (maintained until 17 September) coincided with counts of well over 200. Temperatures fell during 18–24 September to just below 60° F. (average): and low counts followed from then on, despite a very warm fortnight during 1–15 October.

In 1950 (Fig. 2) a rise in weekly average temperature to 65° F. for 4–10 June was followed by a sharp rise in the *Cladosporium* count from much below 100 to near 300. In mid-August the average temperature fell below 60°F.: the counts dropped from well above 100 to 35 and did not rise above 100 again.

The variations above described appear to suggest that the optimum temperature for conidial production in the wild for the strains caught by us is above 60° F. Presumably, in October 1949, conidial production was limited by some other factor than temperature.

Rainfall

During July and August 1949 (when the highest catches were obtained) there was a rainfall deficit of 1·74 and 1·66 in., corresponding with a precipitation equal to 43 and 58 % of the 57-year average, respectively; while during the same months of 1950 there was an excess of 2·12 and 4·69 in., corresponding with precipitations of 170 and 218 %, respectively. Evidently the average humidity at or near ground level was much higher

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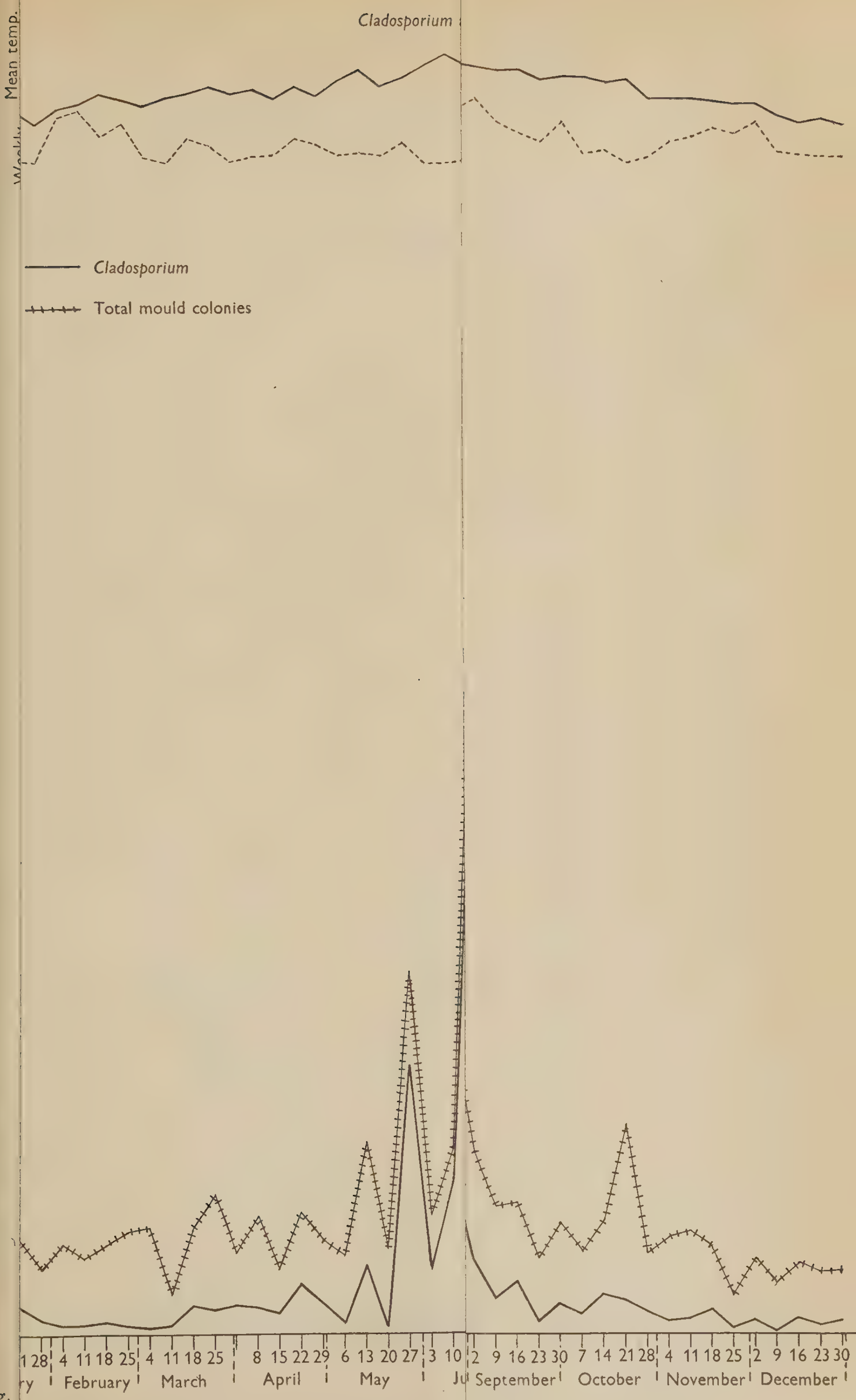
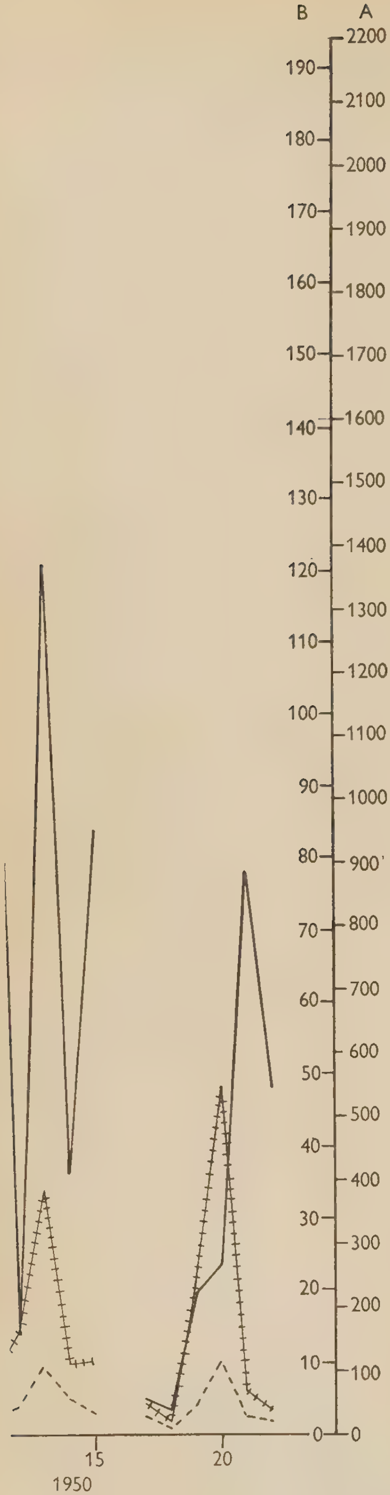
* Weather data from Penylan Observatory (202 ft. O.D., 4¼ miles NNE. of Llandough Hospital and 1½ miles NNE. of the National Museum) by courtesy of the Medical Officer of Health, City of Cardiff.



ig. 2. Incidence of *C. herbarum* at Llandough, Cardiff, 19n to Fig. 1 and text *passim*.



he roof of the National Museum of Wales, ɔ min. Petri plates exposed at Llandough the assumption that deposition proceeded in July–August 1950 than in 1949 when the catch was 90% higher. If 'the production of conidia in Fungi Imperfecti is favoured by the presence of free water or high atmospheric humidity' (Boughey, 1949), then this effect must have been more than offset in *Cladosporium* in 1950 by the lower temperature or by some other factor.

*Plate counts compared with those from elsewhere**

Most workers have used 9 cm. plates but some have used 12 cm.; periods of exposure have varied from 10 to 30 min.; sometimes more than one plate has been exposed at a time; and the kind of apparatus in or on which the plate has been exposed has varied. Comparisons between results can therefore be approximate only. In what follows other workers' data have been recomputed where necessary to a basis of one 10 min. 9 cm. plate a day; weekly counts have been adjusted to a 6-day week and monthly ones to a 26/7-day month.

The highest count for a single day at Llandough (11 August 1949) was 336, which may be compared with 200 obtained in a garden in Copenhagen (5 July 1904; Rostrup, 1908). The highest *weekly* count (1–6 August 1949) was 525, as compared with Copenhagen 460 (in late July 1947; Flensborg & Samsøe-Jensen, 1948), Chicago 258 (in the fall of 1937; Bernstein & Feinberg, 1942), Paris 236 (in July 1948; Vallery-Radot *et al.* 1950) and Örebro 100 (in September 1947; Nilsby, 1949). The highest count for a calendar month (July 1949) was 1478 as compared with Paris 485 (July 1948; Vallery-Radot *et al.* 1950) and Chicago 406 (October 1937; Bernstein & Feinberg, 1942). Our seasonal totals (1949 and 1950, respectively) were 4435 and 2926 as compared with Winnipeg (1949) 3330, and Des Moines, Iowa (1949) 2700 (reported by Harris, 1950), Los Angeles, California (1941–4 average), 2022 (Targow & Plunkett, 1951) and Örebro, Sweden (1946–7) 915 (Nilsby, 1949).

If plate counts alone may be taken as a fair indication of incidence, *Cladosporium* is probably more prevalent at Cardiff than at any other centre from which reports have come.

SLIDE CATCHES OF *CLADOSPORIUM* CONIDIA

Method of counting, and results

We have made counts of *Cladosporium* conidia caught on 'gravity slides' exposed in our standard apparatus (Hyde, 1950) at Cardiff on 73 days, on 65 of which Petri plates also were exposed. Determination, even of small individual conidia, presented no difficulty. We confirmed Brooks & Hansford's (1923) observation that warty conidia greatly predominate in catches thus made from the wild, though not in colonies on artificial media. Nine-tenths of the conidia arrived on our slides in groups (of up to 100) and nearly one-half either as single conidia or in groups of two to six; this grouping was largely unaffected by the process of mounting. We refer to the units in which conidia are caught (and presumably in which they are borne through the air) as units of dispersal. Complete counts on 5 cm.^{2} of each of forty-nine slides exposed on the roof of the National Museum of Wales, Cardiff, 13 June–31 July 1949, showed that 4286 units of dispersal comprised in all 15,553 conidia, an average of 3·62 conidia per unit. The weather records available to us do not support the view that in windy weather the average unit comprises more conidia, though this might perhaps be expected.

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* It is assumed that the name *Hormodendrum* (*Hormodendron*) used in the U.S.A. and elsewhere is a synonym of *Cladosporium*.

Slide counts compared with those from elsewhere

Our counts cover only short periods, but nevertheless in view of the relatively large numbers involved some comparisons seem to be justified. Targow & Plunkett (1951) counted units of dispersal (clumps) and estimated that on the average each unit comprised seven conidia. Their highest count for one calendar month (June 1945) was 94/2 cm.^{2} (=235/5 cm.^{2}) as compared with our 3515 units for 1–31 July 1949 (the only complete month counted) at Llandough. These numbers, however, are small in comparison with those of Durham (1938, 1942, 1946), who reported that his clumps averaged five or six conidia but at times reached forty to eighty, though he did not state any average number of conidia per unit of dispersal and gave his counts as total conidia. He mentions sixteen stations in various parts of the U.S.A. (all inland) at eight of which he had daily catches of 1000–2800/1 cm.^{2} (=5000–14000/5 cm.^{2}) and at the others 500–1000 (=2500–5000/5 cm.^{2}). By contrast, Patterson & Gay (1932) obtained at the most 172/1 cm.^{2} (=860/5 cm.^{2}) per diem only at Baltimore. Our highest daily count (Llandough, 25 July 1949) was 1855/5 cm.^{2}.

Slide and plate counts compared

In Fig. 3 the *actual* day-to-day slide counts, both units and total conidia, for certain periods are shown and compared with *expected* counts (units) which have been deduced from the plate counts on the assumption that one unit of dispersal gives rise to one colony and that such units continue to be deposited at the same average rate throughout the 24 hr.

Series 1 (Fig. 3). Slides exposed in standard apparatus 60 ft. above ground-level on the roof of the National Museum of Wales, Cardiff, 2¾ miles from Llandough Hospital, 13 June–30 July 1949; plates at Llandough Hospital. This series began before the seasonal uprush in the plate counts and continued so as to include the highest peak but one in the season. There is a broad correlation between plate and slide counts, but the latter averaged only 18 % of expectation (based here on the additional assumption that the incidence of *Cladosporium* is the same at the two stations.

Series 2 (Fig. 3). Plates and slides exposed in the same apparatus at Llandough for three 6-day weeks 3–22 July 1950. No apparent correlation. Slide counts (units) averaged 8 % of expectation.

Series 3. As Series 1, but 4–10 December 1950. No apparent correlation. Slide catch 26 % of that expected.

The results of previously published comparisons are conflicting. Harsh & Allen (1945) obtained at San Diego, California slide counts equal to 83 % of expectation based on the corresponding plate counts (one 20-minute plate weekly throughout the year). Harris (1950) reporting on a mould survey for 1949 in the United States remarks on the poor agreement between counts of *Hormodendrum* obtained by the two methods. Targow & Plunkett (1951) obtained at Los Angeles a good correlation between the two, month by month over 4 years, but their actual slide catches over the whole period (expressed as units of dispersal or 'clumps') work out at only 2·7 % of expectation, as compared with our summer value of 8–18 %.

Several explanations of the discrepancy (observed both by Targow & Plunkett and ourselves) between the numbers of units of dispersal *actually* caught on 24 hr. slides and numbers expected from consideration of the results of exposing 10 min. Petri plates are possible. (*a*) These authors suggest that an appreciable number of *Hormodendrum* spores that fall singly are so small as to be overlooked. Having regard to the size range of the conidia we observed in material collected from the wild, we think it improbable that any significant proportion of the conidia on our slides was overlooked.* (*b*) Plates may be more efficient samplers than slides. However, Bourdillon & Lidwell (1948) state that plates are of restricted value for sampling particles below 30 μ in diameter (as most of the units of dispersal concerned must be), and we judge from discussion with Dr P. H. Gregory that slides exposed in our apparatus should be relatively more efficient, not less so. (*c*) Atmospheric turbulence is lower at night than in the daytime and, other things being equal, this would be conducive to a reduced catch; but at the height of summer this would operate over less than half the 24 hr. and would hardly be likely to produce a result of the observed magnitude.

We think, therefore, that the discrepancy is most likely to be due to (*d*) a much higher atmospheric concentration of *Cladosporium* conidia obtaining at the time of exposure of the plates than on the average over the whole of the 24 hr.: this would imply that the conidia were derived from sources in and around Cardiff and that they were being liberated in greater quantities in the morning than on the average during the rest of the 24 hr.

We acknowledge with gratitude the research facilities provided us until September 1950 at Llandough Hospital (Welsh National School of Medicine), and since that date, by the opening of the Asthma and Allergy Research Unit, at St David's Hospital (Cardiff Hospitals Management Committee). The diagrams have been made by Mrs K. F. Adams, B.Sc., who assisted also with the spore counting.

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* Targow & Plunkett's slide catch of *Alternaria* was only 25 % of expectation based on their plates; but in view of the size of *Alternaria* spores the same explanation could hardly apply to them.

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SOME FURTHER DEFINITIONS OF TERMS USED IN PLANT PATHOLOGY

Since the Plant Pathology Committee published a first list of recommended definitions of technical terms ('Definitions of some terms used in plant pathology', *Trans. Brit. mycol. Soc.* **33**, 154–160, 1950), very little comment or suggestion has been received.

The Committee has, however, since minuted its approval of a number of further definitions brought before it from time to time, and these are given below.

Epidemic. Widespread temporary increase in the incidence of an infectious disease.

Epidemiology. Study of the factors affecting outbreak and spread of infectious disease.

Ecoclimate. Climate within a plant community, e.g. a crop.

Focus. Site of local concentration of diseased plants or disease lesions, either about a primary source of infection or coinciding with an area originally favourable to establishment, and tending to influence the pattern of further transmission of the disease.

Infection gradient. Change in incidence of disease with distance from a source of infection.

Necrosis. Death of part of a plant.

Contaminated. Bearing or containing a foreign substance or organism (but not infected thereby).

Index of prevalence. The proportion of stands affected by a disease in a defined area.

Toxicity. The power of acting as a poison.

REVIEWS

Plant Diseases. By F. T. Brooks. 2nd edition. (London: Geoffrey Cumberlege; Oxford University Press. 1953.) xiii + 457 pp., 62 figures. Price 38*s*.

Broadly speaking one can say that plant pathological research, as a thing of national interest, had its beginnings in the first decade of this century. There were of course notable pioneers long before that time, but they were solitary workers and the facilities at their disposal were often meagre. The first World War and its aftermath emphasized the need for more intensive production and for conservation of crop plants, both in the world at large and especially in Great Britain, and this led to a sharp intensification of research in plant disease. Thus by the time that the first edition (1928) of Professor Brooks's well-known text-book appeared, an extensive literature had accumulated and the broad picture of the subject had taken shape. In the twenty-five years that have passed since then research has gone on at undiminished rate, and in Britain at least there was a further expansion as an outcome of the second World War. The time was therefore ripe for a revised edition which would incorporate the mass of newer literature, and it is fortunate for students of plant pathology that Professor Brooks was able to undertake and complete this labour in the few years which elapsed between his retirement and his untimely death.

The second edition of *Plant Diseases* follows the general plan of its predecessor. Though a considerable proportion of the text has been rewritten the sequence of chapters and the mode of treatment are as before. The main emphasis is laid on the identification of specific plant diseases from symptoms and from causal agent, and especially from the latter. Hence the adherence to what some people might call the old-fashioned method of grouping plant diseases of fungal origin according to the classification of the fungi. This is the traditional method, and for general purposes it is not obvious that any one of the alternative methods which have been suggested is an improvement upon the old. The diseases listed and to a greater or less extent described are very many, including practically all which have been recorded on cultivated plants in Great Britain, together with a representative selection of important crop diseases which occur elsewhere. For the majority of those cited the description is very brief and for none is it carried to full detail. The aim is to place the student 'upon the track' of the disease and for a fuller account he must go to a more specialized book or, what is more probable, to the technical journals. For this purpose the text of each chapter is supplemented with an extensive bibliography, and as a measure of the author's industry one may note that, whereas the new edition is longer by about one-fifth than the old, the reference lists and the index are increased by two- or three-fold.

Much the greater part of the book is taken up with the consideration of plant diseases of fungal origin. This is not surprising in view of Professor Brooks's own research activities, but it should also be remembered that fungi still maintain their position as the chief agents of plant disease, certainly in their numbers and probably also in the economic losses which they cause. Nevertheless, one must remark that the treatment of virus diseases is distinctly brief in relation to their importance, while the chapters entitled 'Non-parasitic Diseases' (including deficiency diseases) and 'Fungicides' give little more than honourable mention to two fields of research which have been particularly active in recent years. The author is well aware of these omissions and directs the reader to the relevant special text-books. The breeding of plants for resistance to disease is dealt with merely incidentally, and the young student will look in vain for any account of the methodology of plant disease study.

It might be asked at this point whether, in view of the ever-increasing tendency to specialization in plant pathological research, a general text-book is any longer practicable. Research on fungicides, on viruses, on mineral deficiencies, and so on, requires specialized training of the worker; furthermore, the orientation of research (by finance, in the last resort) is coming more and more to the pathology of specific crops, such as cereals, vegetables or more narrowly still, of potatoes, cotton, etc. The 'general' plant pathologist thus seems to be following the 'general' botanist in the direction of extinction. One may suggest a suitable answer in this form, that the general type of text-book is still possible, and is in fact highly desirable, so long as it maintains a standard of generality and does not attempt to set out all the intricacies and all the contradictions or apparent contradictions which are so liable to occur in the literature of a rapidly developing science. Some of the more voluminous treatises have erred in this respect. To the young student or the general reader the mass of detail is forbidding and often perplexing. At the other extreme the specialist worker has little use for text-book detail because he must in any case read the original literature bearing on his work, and having collated this in the light of his own experience he is in a much better position to frame conclusions than is the text-book writer who lacks that special experience. The essence of a general text-book then is that, while it covers the ground, it should remain general and brief. *Plant Diseases* is a shining example of the brief concise type of general text-book; it is eminently readable to the student, and the specialist will use it as a ready means of keeping broadly in touch with the subject as a whole.

Brevity and clearness of description are qualities which are inevitable in Professor Brooks's writings. He writes as he spoke and that was forcefully, to the point, without repetition or wordiness or superfluity of ornament. To those of us, of the older generation, who enjoyed his friendship over many years, the new edition of *Plant Diseases* is yet one more illustration of his undoubtedly great capacity, so often shown round the committee table, for the orderly handling of data and for quickness and lucidity of presentation.

W. BROWN

Les Champignons. Tome I. By F. MOREAU. (*Encyclopédie Mycologique*, XXII. Paris: Paul Lechevalier. 1953.) 940 pp., 456 text-figures. Price Fr. 11,000.

This first volume of *Les Champignons* is concerned largely with the biology of fungi. Following an introductory chapter on the general characteristics of fungi the subject-matter is treated in two sections. Part I, 'Physiologie', begins with chapters on the occurrence of saprophytic fungi in nature and on the use and maintenance of pure cultures. It continues with several chapters on physiology, including discussions on the heterotrophic condition, the nutrition, metabolism and enzyme systems of fungi. Concluding this section are chapters on the general features of parasitism, on symbiosis, and finally on diseases of plants and animals and their control. Part I has many references to the economic applications of fungi, e.g. the uses of fungi as food, in medicine, including a chapter on fermentation in the service of man.

Part II, 'Morphologie et Développement', opens with an account of the morphology of the fungus thallus and continues with chapters on the cytology of living and fixed material, staining methods, reproduction, liberation and dispersal of spores, spore germination. Then follows an historical account of the development of knowledge of sexuality in fungi, which leads to a consideration of heterothallism, genetics and evolution of sexuality.

An extensive bibliography is scattered through the book, with a list of key references at the beginning and a separate list at the end of each chapter or chapter section. The index is included at the end of Tome II, a great mistake.

There is an enormous amount of information in this book, as its size suggests, and one cannot but admire the patience of Prof. Moreau in bringing together this mass of material, covering such a wide field, and congratulate him on this achievement. The size of the book is likely to prove a serious disadvantage, however, for at 11,000 francs, a truly monumental price, few will be able to afford it. From the point of view of the teacher, the book falls between two stools. It is too extensive for first courses in mycology, while advanced students will prefer to consult books devoted entirely to a consideration of the particular fields, e.g. plant pathology, fungal physiology, in which they are interested. As a general reference book, however, it will form a useful addition to mycological literature.

C. J. HICKMAN

Les Agaricales. Tome II: Russulacées, Hygrophoracées, Gomphidiacées, Paxillacées, Boletacées. By P. KONRAD and A. MAUBLANC. (*Encyclopédie Mycologique*, XX. Paris, P. Lechevalier. 1952.) 202 pp. Price Fr. 2200.

The long-awaited second volume of Konrad and Maublanc's useful work, *Les Agaricales*, has now appeared, after a delay of four years due largely, no doubt, to the untimely death of M. Konrad. The first part of the work, dealing with the family Agaricaceae only, appeared in 1948 and was reviewed in these *Transactions*, Vol. **33**, p. 190, 1950. The second part treats in similar fashion the remaining families of the order Agaricales, as enumerated in the subtitle. This separation of the Agarics into several families, and the inclusion of the Boletaceae, may be unfamiliar to some of the older mycologists, but is in accordance with modern views. The Russulaceae (*Russula* and *Lactarius*) and the Hygrophoraceae (with the single genus *Hygrophorus*) are now generally considered sufficiently distinct, on grounds of anatomy, from other agarics to be worthy of equal family rank. Similarly, the Gomphidiaceae and Paxillaceae appear to have their nearest allies in the Boletaceae, and are accorded equal rank with that family. Mention is made of suggested relationships between the Russulaceae and certain genera of Hypogeal Gasteromycetes, but the authors confine their notes to the lamellate forms.

Since the publication of the first volume many new records have been added to the European fungus-flora, and there have been several important monographs and revisions, as well as some good illustrated popular works. References to these are included in the present volume, and they have necessitated a long list (pp. 147–191) of Addenda and Corrigenda to the first volume. A few important papers, such as Moser's revision of *Cortinarius*, section *Phlegmacium* and Schaeffer's *Russula* monograph appeared too late to be included in the references under species, but they are mentioned at the end. The work is completed by an index to the whole. E. M. WAKEFIELD

Introductory Mycology. By C. J. ALEXOPOULOS. (New York: John Wiley; London: Chapman and Hall. 1952.) 482 pp., 187 figures. Price 56*s*.

If de Bary's *Morphology and Biology of the Fungi*, 1887, is taken as the standard, a satisfactory modern general text-book of mycology has yet to be written, but students and teachers should not let this omission obscure the many excellent features of the latest introduction to mycology. The object of *Introductory Mycology* is an 'orderly presentation of certain fundamental facts on the structure and classification' of fungi for the student who knows nothing about these organisms, and in this the author may be judged to have succeeded.

The general treatment is the orthodox taxonomic approach, and after an introductory chapter, Bacteria to Basidiomycetes are dealt with in a series of fifteen chapters which are followed by a page and a half on lichens, a useful glossary, sources of the illustrations, and a general index. The presentation is up-to-date. Sparrow's classification of the Phycomycetes is accepted and the Fungi Imperfecti are adequately introduced by a chapter inserted after the Ascomycetes.

The illustrations ('Art work'), mostly taken from many familiar and less familiar sources, have all been drawn or redrawn by Mrs Sun Huang Sung which gives the figures an unusual unity and their consistent arrangement in groups to emphasize the life cycles of the various fungi illustrated is a helpful innovation. The fact that no clue is given of the scale of any figure and the juxtaposition of figures of very different magnifications is to be deplored, for in the reviewer's experience it is always most difficult to give inexperienced students a true idea of microscopic size and to induce them to maintain consistency in the scale of their records of microscopic detail. (To those who have the same difficulty it may be noted that to keep a supply of *Lycopodium* spores always available in the elementary laboratories provides a cheap and useful approximately 30 μ reference standard for both 'low-' and 'high-'power microscope work by the incorporation of a few spores in every temporary mount.) However, the book is to be warmly recommended both for those for whom it was written and as revision reading for those who are more advanced. G. C. AINSWORTH

Le Bactériophage. (Paris: Institut Pasteur. 1953.) 318 pp. Price Fr. 1675.

In the summer of 1952 the first international colloquium to be devoted to bacterial viruses was held at the *Abbaye de Royaumont* near Paris, and the papers presented there have now been published, with commendable promptitude, as a special number of the *Annales de l'Institut Pasteur.* The twenty-nine papers, twelve in French and seventeen in English, vividly portray the enthusiasm and energy with which new techniques which give results with a rapidity and quantitative reproducibility rare in biology are being brought to bear on the study of these fascinating agents,

The individual papers differ in character; some report restricted pieces of research, others summarize current views on such varied problems as the structure of virus particles, the origin and fate of their components, the mechanism of infection, the 'genetics' of bacteriophage, and the methods whereby phage can be artificially induced in *Bacillus megaterium.* It is refreshing that lysogenic bacteria are again exciting much attention, both because of their potentialities in providing clues to the origin of viruses and because work during recent years has been confined too narrowly to the T phages of *Escherichia coli.* The way in which these dominate the subject is amply shown by the fact that 20 of the papers are concerned with them.

It is invidious to select any paper for special mention, but invidious one must be and commend Hershey's succinct review entitled 'Intracellular phases in the reproductive cycle of bacteriophage T2'. It tells of the tadpole-like particles attaching themselves to bacteria by their tails, the passage of their contents into the host, while their membranes remain outside, the disappearance of the particles as recognizable entities, then the reappearance, first of non-infective tail-less particles, followed by the infective tadpoles before the host cell lysis. Although many details remain to be filled in, a clear outline picture is drawn, and while drawing it Hershey distinguishes between facts and interpretations more clearly than do some of the other contributors. F. C. BAWDEN

La description des champignons supérieurs. By MARCEL JOSSERAND. (Paris: Paul Lechevalier. 1952). 338 pp., 232 figures. Price Frs. 3500.

Any book by M. Josserand would be sure of a welcome and he is well qualified to produce a work designed to assist mycologists and especially agaricologists in the use of appropriate language. The meaning of many terms in current use is far from clear. Sometimes these terms are used in conflicting senses. This book chiefly consists of a vocabulary of most, if not all of the terms used in describing agarics. Reasoned arguments are brought forward for the adoption, retention or rejection of many words we are familiar with, but are apt to use with doubtful accuracy. The authority of the author should ensure that there will be no excuse for such misuse of mycological terms. Although the author is referring to the French language, most of the words are very similar in all languages so his views apply to all. This part of the book is preceded by a very useful and practical guide to the technique for observing microscopical characters. The price is high, like all such books nowadays. It is no use protesting while the cost of printing has reached such fantastic levels. A. A. PEARSON

KLIC Agaricalium Europaeorum Clavis Dichotomica. By ALBERT PILÁT. (Prague, 1951).

On p. 238 of vol. **35** of these *Transactions,* we inadvertently assumed that this excellent work, written in Czech, had received state support. Indeed it seemed impossible that private enterprise would be responsible for such a costly undertaking. We were wrong. Dr Pilát has written as follows: 'The book was published in an edition of 3300 copies and the price was fixed according to the cost in the normal way. The whole edition was sold out within two months.'

A. A. P.

Medical Mycology. An Introduction to its Problems. By G. C. Ainsworth. (Sir Isaac Pitman and Sons, Ltd. 1952). 105 pp., 8 plates. Price 15*s.*

This little book is an authentic, readable, and useful presentation of some of the principles involved in the study of mycoses and the fungi which cause them. It is not intended to serve as a text-book of medical mycology, but rather, to define and clarify certain specific problems fundamental to the subject. This is accomplished by critical discussions of selected mycoses which best illustrate these problems.

Thus the dermatophytes, which represent the most numerous group in medical mycology and whose species, according to the author, have appeared under some 1000 binomials, are appropriately used to illustrate the problems of taxonomy and nomenclature. After a brief review of their importance and of certain epidemiological aspects of the superficial mycoses, the ringworm fungi themselves are considered. Their parasitism, their variability (both spontaneous and induced) and attempts to develop a sound and natural classification are reviewed. The practical applications of the International Rules of Botanical Nomenclature are pointed out.

Likewise, some of the epidemiological and etiological problems presented by *Candida albicans* and other species of *Candida* are cited to illustrate the difficulties met in evaluating the presence of fungi in the respiratory tract. When *C. albicans*, *Aspergillus fumigatus*, or certain other fungi ordinarily known as saprophytes, are found in sputum are they merely contaminants, or do they play secondary or primary roles in the causation of pulmonary disease?

The problem of multiple causation is exemplified by discussions of mycetoma or 'Madura foot' (for which thirty-five etiological agents are tabulated) and by chromoblastomycosis, a well-defined syndrome which is caused by any one of three curiously interrelated fungi. The dimorphic character of the fungi pathogenic for man is best developed in those fungi causing systemic mycoses, and the author discusses in this connexion coccidioidomycosis, sporotrichosis, the North and South American blastomycoses, and histoplasmosis. A pertinent discussion of the probable sources of infection for these mycoses is included.

A chapter on serology is introduced with a presentation of some underlying principles of immunology and a review of a small segment of the large literature on the antigen-antibody reactions of the mycoses. Unfortunately, serologic procedures are still largely within the realm of experimental laboratory studies rather than well standardized diagnostic procedures. The role of fungus spores as allergens is presented historically and in terms of modern studies of specific fungus allergens, the occurrence of mould spores in air, and occasional instances of occupational exposures. Finally, there is a useful chapter on those fungi, principally some of the mushrooms, which produce toxins and are responsible for cases of mushroom poisoning. It outlines types of toxins and the symptoms of poisoning they cause.

The book contains very few errors. There is one reference to "acid fast granules" of *Actinomyces israeli* which may lead to some confusion since acid fastness is a characteristic of Nocardia rather than of Actinomyces. It might be pointed out also that there are several important exceptions to the generalizations in Table II reproduced from Henrici's valuable 1940 paper,

This book is a stimulating introduction to medical mycology. It places in good perspective some of the fundamental and fascinating problems peculiar to this field.

CHESTER W. EMMONS.

THE BRITISH MYCOLOGICAL SOCIETY TRANSACTIONS

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5	*m*	*m*	*m*	—	—
6	*m*	*m*	(20*s.*	)	—
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